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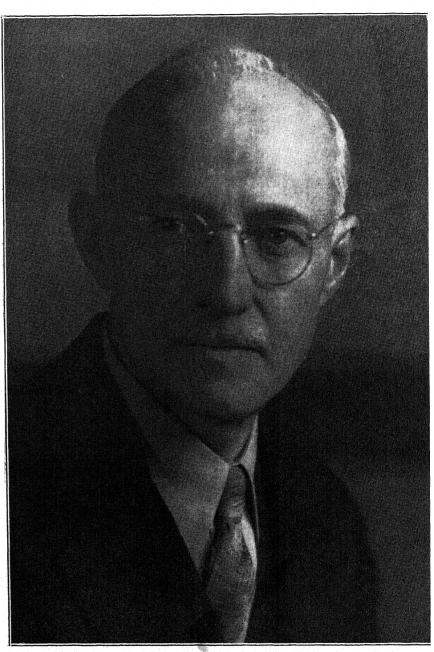
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WILLIAM TITUS HORNE 1876-1944

WILLIAM TITUS HORNE 1876–1944

T. E. RAWLINS

We were very sorry to learn of the death of Professor William Titus Horne on April 12, 1944. In his passing his former students and colleagues have lost one of their finest friends.

Professor Horne was born November 8, 1876, in Kankakee, Illinois. He graduated from the University of Nebraska in 1898, and later had a year of graduate work at Columbia University. From 1904 to 1909 he was connected with the Agricultural Experiment Station of the Cuban Government, the last three years as Chief of the Division of Plant Pathology. In 1909 he joined the staff of the Plant Pathology Division of the University of California at Berkeley where he took over the teaching of plant pathology and continued this important work until 1928. During this period his keen interest in the welfare of his students encouraged many of them to take up plant pathology as a life work. While in Berkeley he and his students worked on a number of important and difficult problems. Among them were brown rot and virus diseases of stone fruits, strawberry virus diseases, olive diseases, Armillaria root rot and fig spoilage. Numerous important publications resulted from this work.

In 1928 Professor Horne moved to the Citrus Experiment Station at Riverside, California, where he began research on diseases of the avocado and certain other subtropical fruits, a field in which he had always been particularly interested. He was also very successful in this endeavor as is indicated by his being honored with one of the first service medals presented by the California Avocado Society in 1939 and by his election to the presidency of the Pacific Division of the American Phytopathological Society in 1938–39. During this period he made numerous contributions on the diseases of the avocado and other subtropical fruits. His bulletin, "Avocado Diseases in Southern California," is probably the most complete and reliable publication on avocado diseases and is a final excellent chapter in an outstanding life of service.

He was author or co-author of the following publications:

A new species of Lembosia (Lembosia Rolfsii sp. nov.). Bull. Torrey Bot. Club 32: 69-71. 1905.

El Minero de las hojas y otras plagas del cafeto. (Coffee leaf miner and other coffee pests.) In English and Spanish. Estac. Cent. Agron. Cuba Bull. 3. 1905. (With Mel. T. Cook.)

Fumigation de las plantas citricas. (Fumigation of citrus trees.) Bol. Ofic. Sec. Agr. Indust. y Commer. Cuba 2(5): 325. 1907.

Report on the cocoanut disease known as bud rot or heart rot. Bol. Ofic. Sec. Agr. Cuba 3(1): 1-5. 1907.

Pudricion del Cogollo y otras enfermedades del cocotero en Cuba. (The bud rot and some other cocoanut troubles in Cuba.) Estac. Cent. Agron. Cuba Bull. 15. 1908. An injury to pine trees in Cuba, caused by Dioryctria Sp. and other Lepidoptera. Estac. Cent. Agron. Cuba Rpt. (1905–1909) (English Ed.) 2(2): 147–149. 1909. Also in Spanish. (With J. S. Houser.)

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Resistance of peach hybrids to an obscure disease in southern California. Jour. Heredity (With Geo. P. Weldon and E. B. Babcock.) 17: 98–104. 1926.

Notes on fruit decays of the feijoa (Feijoa Sellowiana Berg.) Year Book Calif. Avocado Assn., Rept. 1927: 31-33. 1927.

Notes on the experimental inoculation of avocado seedlings with the pear blight organism, Bacillus amylovorus (Burr.) Trev. U. S. Dept. Agr. Plant Disease Reporter 12: 7.

The improved eastern blueberry in California. Madrono 1: 179-183. 1928.

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Carapace spots (avocado). Year Book Calif. Avocado Assn. 1929: 129. 1929.
Avocado water injury. Year Book Calif. Avocado Assn. 1929: 33-34. 1929.
The avocado disease called sun-blotch. Phytopath. 21: 235-238. 1931. (With E. R.

Parker.)

"Buckskin," a destructive graft-infectious disease of the cherry. Phytopath. 21:
331-334. 1931. (With T. E. Rawlins.)

Monthly Bull Calif. State Dept. Agr. 20: 447-454.

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1931. (With E. R. Parker.)

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A mosaic of the fig in California. Phytopath. 23: 887-896. 1933. (With I. J. Condit.) Avocado diseases in California. Calif. Agr. Exp. Stat. Bull. 585. 1934. The control of Dothiorella rot on avocado fruits. Calif. Agr. Exp. Stat. Bull. 594. 1935.

(With D. F. Palmer.)

Dothiorella fungus in frozen avocado trees. Calif. Avocado Assn. Year Book 1937: 154-155. 1937.

Nematode infestation of olive roots. Phytopath. 28: 756-757. 1938. (With Ira J. Condit.)

THE INFLUENCE OF FUNGI ON STORAGE, ON SEED VIABILITY AND SEEDLING VIGOR OF SOYBEANS¹

IAN W. TERVET

(Accepted for publication May 7, 1944)

Except for the information given by Dickson (2), there are few published reports on the occurrence of bacterial and fungus pathogens on soybean seed. Some fungi have been isolated from soybean meal, silage, or other products derived from soybeans. From moldy soybean cakes, species of Aspergillus, Penicillium, Monilia, and Sphaerella were obtained (7), while Byssochlamys musticola Naoumoff and Kiryalava was isolated from nonacid soybean silage (6). Ramstad and Geddes (8) report several fungi in soybeans damaged in storage, while Milner et al. (5) have shown that soybeans injured by frost have more seeds infected by fungi, principally Alternaria and Fusarium, than uninjured seeds.

Satisfactory storage of soybeans to avoid heating and spoilage requires, beside suitable storage conditions, that the beans be maintained at a low moisture content. Burlison et al. (1) have shown that deterioration of soybean seed in farm storage is most rapid when the seeds are allowed to absorb moisture, and potential germination decreases rapidly under such a condition. Ramstad and Geddes (8) also observed that the viability of soybeans decreased rapidly under conditions favoring the growth of microorganisms, and that soybeans suffered damage when stored at high moisture levels at room temperature, even though heating did not occur.

The microflora was studied in soybean seed grown in 19 counties of Minnesota in 1942. Studies on the relation of fungi to heating and to viability of soybeans also were initiated.

MICROFLORA OF FROST-INJURED SOYBEAN SEED

Much of the soybean seed harvested in Minnesota in 1942 was injured by frost, the extent of the injury on different samples varying greatly. Many samples were green and shrivelled, the germination of the seed being generally poor. Surface-disinfected soybean seeds² were plated on potato-dextrose agar. Frost-injured seeds were more likely to be infected by fungi and bacteria than noninjured seeds. Alternaria predominated, with Fusarium next in frequency on the seeds. Aspergillus spp. occurred on occa-

¹ Paper No. 2161, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

Cooperative investigations between the Division of Plant Pathology and Botany and Agricultural Biochemistry. The writer expresses his deep appreciation to Dr. W. F. Geddes and Mr. Max Milner, of the Division of Agricultural Biochemistry, for preparing many of the soybean samples, for supplying all of the moisture data as well as the results obtained in the adiabatic studies given in this report, and for their interest and enthusiasm during the course of the work.

² Seeds dipped in 70 per cent alcohol, immersed in mercuric chloride (1:1000) for 2 min., washed in water for 5 min., rinsed in calcium hypochlorite 1.3 per cent for not less

than 5 min., then plated directly from the latter solution on agar plates.

marc by t y the g the to enci ies tha on the sional samples, in one case 25 per cent of the seeds being infected. Rhizopus nigricans Ehr., Chaetomium sp., Cephalothecium roseum Corda, Trichoderma viride (Pers.) Fr., and some nonsporulating fungi were found. Bacteria were present in most severely shrivelled samples and not infrequently developed from seeds that yielded also Alternaria and Fusarium. Indeed, it was not uncommon for seeds to give rise to two different colonies of fungi, or a fungus and bacterium. A summary of the microflora analysis is in table 1, the seed samples being grouped on a regional basis.

Two series of soybean samples combining seed lots with different percentages of frost-injured seeds were analyzed for microflora and for the effect of seed treatment on the germination of the seeds. One set of samples was obtained from Illinois through the courtesy of the Regional Soybean Laboratory, Peoria, Illinois; the second sample was of Minnesota origin. The Illinois samples were less severely injured by frost, and on the average

TABLE 1 .- The occurrence of microorganisms on soybean seeds injured by frost

Origin	No.		Frost- injured				
of samples	of of		Alter- naria	Fusa- rium	Miscel- laneous fungi	Bac- teria	beans (per cent)
South East Minnesota	45	Av. 35.9 Range 0-85	28.9 9–53	19.0 2–41	2.2 0–12	16.0 1–82	33.0 4–78
South West Minnesota	24	Av. 43.7 Range 2–88	33.2 1–71	12.1 0-44	$\begin{array}{c} 1.6 \\ 0-25 \end{array}$	$14.3 \\ 0-65$	22.3 0–58
Central Minnesota	11	Av. 24.0 Range 4-69	56.3 29–81	10.0 0-24	0 0	$\begin{array}{c} 15.2 \\ 1-46 \end{array}$	40.3 8–67

had fewer infected seeds. Alternaria was the most common fungus in the Minnesota samples, Fusarium being less abundant; in the Illinois samples Alternaria and Fusarium were found in equal amounts (Table 2). A non-sporulating fungus, resembling that mentioned by Johnson and Koehler (4), was commonly associated with a purple spotting on seeds from the Illinois samples. The number of seeds free from infection decreased as the percentage of frost-injured seeds increased. Germination of the seeds varied with the extent of frost injury, the lots with least injury germinating better than those with more injured seeds. Treatment of the seeds with maximum adhesive dosage of Arasan, 50 per cent tetramethylthiuram disulphide, consistently increased the total stand and the percentage of vigorous plants as measured by the number of seedlings with expanded first leaves.

THE MICROFLORA OF SOYBEANS IN RELATION TO LOSS IN VIABILITY AND HEATING OF SOYBEANS

Soybeans with a high moisture content heat rapidly in storage and at the same time the respiration of the sample also is accelerated. It has been suggested that microorganisms play an important part in the high respiratory rates associated with heating (8), but no detailed analysis of the microflora of high-moisture-content beans during storage is available. Attempts were made to determine the changes in microflora associated with increasing moisture content of the beans and with increasing storage temperatures.

The Minnesota lot of soybeans, with 55 per cent frost damage (Table 2), had a moisture content of 6.6 per cent after storage at 2° C. for 2 months. A sample of this lot of beans was conditioned to 17.6 per cent moisture content by placing the seeds in a humidifying chamber at room temperature for 4 weeks, the seeds then being stored at 2° C. for 5 days, and thereafter placed in a Dewar flask. The seeds were very moldy after 1 month's storage

 $\begin{tabular}{ll} TABLE 2. \end{tabular} \begin{tabular}{ll} TABLE 3. \end{tabular} \begin{tabular}{ll} TA$

Seed	Perc		eds infed nd bacte	eted with t	fungi		treated Arasan		s not ated
source and frost damage (per cent)	Sterile	Alter- naria	Fu- sarium	Miscel- laneous fungi	Bac- teria	Seeds germi- nat- ing	Plants with first leaves ex- panded	Seeds germi- nating	Plants with first leaves ex- panded
3.5						Pct.	Pct.	Pct.	Pct.
Minnesota 10.3 13.3 21.0 21.0 26.0 41.0 55.0	70 77 50 50 49 42 27	25 18 30 20 27 39 40	1 5 8 6 11 3 8	1a 0 1a 0 0 1 2a	3 0 1 24 13 15 23	62 51 52 33 28 23 6	94 94 87 79 93 87 67	54 41 39 26 20 14	89 88 77 73 85 71 0
Illinois 3.3 6.4 7.5 10.5 12.3 18.0	85 93 77 56 84 57	0 6 6 10 1 12	4 1 0 0 15 25	2b 0 2 2 0 0	9 0 15 32 0 6	81 80 69 59 60 46	97 92 91 92 87 87	75 73 63 48 44 31	95 96 92 90 84 84

a Rhizopus sp., Chaetomium sp., Aspergillus sp.

in a humidifier. Two hundred surface-disinfected seeds were plated on potato-dextrose agar. Only 6 per cent of the seeds were free from internal fungi, as opposed to 27 per cent sterile seeds in the lot with a moisture content of 6.6 per cent. Also, 66 per cent of the high-moisture-content seeds gave rise to Aspergillus spp., as opposed to 1 per cent in the low-moisture seeds, a very great increase in the prevalence of this fungus at the expense of other fungi and bacteria.

The seeds in the Dewar flasks were allowed to undergo adiabatic heating in an apparatus similar to that described by Ramstad and Geddes (8). In 21 days the temperature rose to 46.9° C. from an initial temperature of 22° C. The beans were then brown and free from superficial mold growth. These seeds were surface-disinfected and plated on potato-dextrose agar, and

b Nonsporulating fungus.

98 per cent were free from internal fungi, while 2 per cent produced colonies of bacteria.

Samples of 1942 Illinois soybeans with an initial moisture content of 6.51 per cent were conditioned to a higher moisture level by placing them in a humidifier. When the seeds reached a moisture content of 8.65 per cent after 4 days in the humidifier a sample was removed and placed in a refrigerator at 2° C., where it remained for $6\frac{1}{2}$ weeks, until the storage experiments were begun. The rest of the seeds were kept in the humidifier for 30 days, their moisture content at the end of that time being 12.95 per cent. These seeds were then kept at 2° C. for 3 weeks. A sample was removed, sprinkled with water, thoroughly shaken and replaced in the refrigerator for 2 days,

TABLE 3.—The effect of adiabatic aerated storage on the microflora and viability of soybeans

		No.	Micro	oflora of	seeds	Seeds t with A		Seed: trea	
Moisture content of seeds	Storage tempera- ture	days in adia- batic stor- age	Sterile	Asper- gillus	Misc. fungia	Seeds germi- nating	Plants with first leaves ex- panded	Seeds germi- nating	Plants with first leaves ex- panded
Pet. 6.51 8.65 12.95 21.2	Degrees, C. 29.4 37.05 44.4 49.3 53.7 55.1 54.9 59.7 67.3 77.0	0 0 0 0 3 5 7 8 10 12 13 15 17	97 97 91 89 84 73 36 60 84 87 91 100 100	0 0 4 3 5 17 53 36 14 11 4 0	3 5 8 11 10 11 4 2 2 5 0 0	Pet. 95 95 85 86 72 68 41 0 0 0	Pct. 92 98 82 78 78 0 0 0 0	Pet. 93 92 71 78 73 52 30 0 0 0 0 0	Pct. 76 89 11 4 12 48 17 0 0 0 0 0

^a Mainly Chaetomium sp., Cephalothecium roseum. Rhizopus nigricans, Cunninghamclla echinulata, and Penicillium sp.

the final moisture content of the seeds of this sample being 21.2 per cent. Four samples with moisture levels varying from 6.51 to 21.2 per cent (Table 3) were obtained by these methods. Plating of 200 seeds of each of these samples on potato-dextrose agar, after surface disinfection, showed that the higher-moisture samples had slightly more infected seeds than the low-moisture samples.

Ten lots of beans with a moisture content of 21.2 per cent were placed in bottles of approximately 300 cc. capacity, about 100 g. of beans in each bottle. These samples were then placed in the adiabatic storage chamber. The bottles and flask were continuously aerated, approximately 8 liters of air per 24 hours at start of experiment, the air being passed through a humidifying solution in order to maintain the moisture content in the seeds.

At intervals (Table 3) bottles of soybeans were removed from storage and the beans immediately analyzed for microflora and tested for viability. Two hundred seeds were plated, and duplicate 100-seed rows of nontreated seeds and of seeds treated with the maximum-adhesion dose of Arasan were planted in sand in a greenhouse at 70° F. Stand counts were made 10 days after planting.

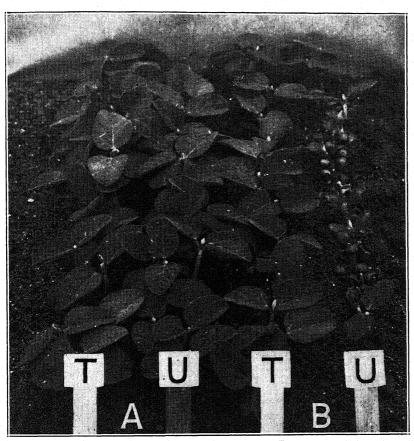


Fig. 1. Effect of seed treatment on stand and vigor of soybean seed lots. A. Plants from low-moisture-content seeds (6.51 per cent), treated with Arasan (T) and untreated (U). B. Plants from high-moisture-content seeds (21.2 per cent) treated with Arasan (T) and untreated (U).

The beans heated rapidly, a temperature of 37.05° C. being reached in 5 days, and 49.3° C. in 8 days, with a maximum of 77.0° C. after 19 days. During the first week in storage, the percentage of noninfected seeds decreased from 89 per cent at room temperature (\pm 22° C.) to 36 per cent at 44.4° C. Coincident with the rise in temperature, the number of seeds infected with Aspergillus spp. increased rapidly from 5 per cent at room temperature to a maximum of 53 per cent at 44.4° C. Beans sampled on the 8th day of storage, when the temperature had reached 49.3° C., had fewer

infected seeds; and by the 15th day at a temperature of 59.7° C. no fungi were found. At the favorable temperature, the predominant fungi were Aspergillus species, the following group species (9) being most common: A. glaucus, A. flavus, and A. ochraceus, while A. niger and A. fumigatus were less frequently found. Several other fungi were also present on the seeds—Cephalothecium roseum Corda, Cunninghamella echinulata Thaxter, Rhizopus nigricans Ehr., Chaetomium sp. and Penicillium sp.

The viability of seeds decreased with increased moisture content of the seed and with rising temperature in storage. Seeds with moisture contents of 6.51 and 8.65 per cent germinated above 90 per cent, with 76 and 89 per cent respectively of the seedlings producing their first leaves within 10 days of planting (Table 3). At a moisture content of 12.95 per cent, 71 per cent of the seeds germinated but only 11 per cent of the plants were vigorous. At 21.2 per cent moisture, 78 per cent of the seeds germinated, but only 4 per cent of the seedlings had produced first leaves 12 days after planting. Treatment of this high-moisture-content seed with the maximum-adhesion dose of Arasan increased the germination to 86 per cent and the number of seedlings with first leaves to 78 per cent (Fig. 1). The viability of seed stored in the adiabatic respirometer decreased rapidly, from 78 per cent at the beginning of storage to 30 per cent at the end of 7 days, and 0 per cent on the 8th day and thereafter. The seeds in storage generally gave rise to stunted plants, except one lot that had been stored for 5 days and in which 48 per cent of the seedlings were vigorous. The reasons for the occurrence of such a high proportion of vigorous plants in this single sample were not determined, but it is possible that the variability of results could be considered as experimental error, although the duplicated rows of seedlings were very similar.

In general, applications of the maximum-adhesion dosage of Arasan to the seeds just prior to planting increased the total stand and resulted in a marked decrease in the percentage of stunted plants, indication that the poor development of the seedlings was due to the presence of microorganisms on the seed (Table 3 and Fig. 1).

EFFECT OF HIGH MOISTURE CONTENT ON THE MICROFLORA AND VIABILITY OF SOYBEANS

Three samples of 1942 Minsoy soybeans were conditioned to different moisture contents by adding water to the air-dried beans, thereafter frequently shaking the samples. The beans were stored at room temperature in quart jars with tight-fitting lids, each jar containing 1 lb. of seeds. The moisture contents of the beans after conditioning were 7.9, 13.3, and 20.4 per cent, respectively.

Six days after the water had been added, 200 seeds of each lot of beans were plated on potato-dextrose agar after surface disinfection, and duplicated 100-seed rows of seed from each sample, untreated and treated with the maximum-adhesion load of Arasan, were planted in sand in a greenhouse

at 70° F. Similar tests were made 13, 25, and 34 days after the initiation of the experiment. The low moisture sample remained fairly constant throughout the storage period, no major changes occurring in the microflora. While some fluctuation in the percentage germination was apparent, the differences were not excessive and probably resulted from variations in greenhouse conditions. The microflora of the second soybean sample, held at 13.3 per cent moisture content, did not change greatly during the first 25 days, but after 34 days bacterial count was high. Germination of this sample decreased during the storage period, varying from 64 per cent after 6 days' storage to 29 per cent after 34 days. In contrast, the high moisture sample changed materially during storage in the number of infected seeds

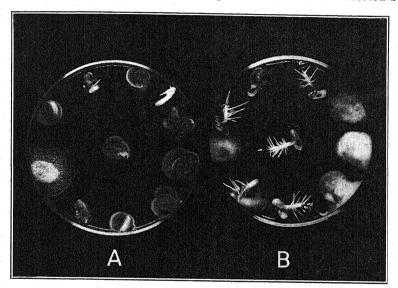


Fig. 2. Microflora of soybean seed. A. High-moisture-content seed with Aspergillus spp. predominant. B. Low-moisture-content seed with Alternaria sp. predominant.

present and in the marked decrease in viability of the seeds. The number of non-infected seeds decreased from 64 per cent after 6 days' storage to 1 per cent after 34 days' storage. Species of Aspergillus and Penicillium were obtained most frequently from infected seeds, about two-thirds of such seeds producing Aspergillus spp., principally A. glaucus, A. ochraceus and A. flavus, while one-third of these seeds were infected with Penicillium sp. (Fig. 2). Germination dropped rapidly, from 46 per cent after 6 days' storage to 2 per cent after 34 days. Seed treatment increased the stand (Table 4).

FUNGI IN RELATION TO LOSS IN VIABILITY OF SOYBEAN SEED

Many fungi were obtained in the microflora studies; and a survey of their effects on the viability of soybeans was made. Isolates of Aspergillus flavus, A. ochraceus, A. glaucus (5 distinct isolates), A. fumigatus, and 3 unidentified species of Aspergillus, Penicillium sp., Alternaria sp., Chaetomium

TABLE 4.—Changes in microflora and viability of Minsoy soybean seeds resulting from storage of seeds at different moisture contents

Microflora,		M	oistur	e conte	ent of	beans	and nu	mber	days ir	stora	ge	
seed viability, and seedling		7.9 p	er cent	; .		13.3 1	er cen	t		20.4 p	er cen	t
vigor	6	13	25	34	6	13	25	34	6	13	25	34
Microflora		P	ercent	ages o	f seed	s infe	cted w	ith mi	croorg	anisms	3	
Sterile Alternaria Aspergillus and Peni-	68 22	72 18	77 16	71 14	79 10	66 16	67 20	44 14	64 23	16 12	7 17	1 25
cillium Bacteria Misc. fungi	0 4 6	0 5 5	0 7 0	1 14 0	1 6 4	6 4 9	$\begin{array}{c c} 1\\12\\0\end{array}$	1 41 0	2 5 6	65 0 8	74 0 2	68 6 0
Seed viability				Perc	entag	es of s	eeds ge	ermina	ting			-
No treatment Arasan-	73	60	67	53	64	54	46	29	46	18	13	2
treated seed	74	58	76	70	61	59	66	37	57	50	20	5
Seedling vigor			Per	centag	es of p	lants	with fir	rst leav	ves exp	anded		-
No treatment Arasan-	93	93	88	89	95	83	63	84	87	72	23	0
treated seed	95	86	92	95	90	88	83	85	93	86	25	10

sp., Cephalothecium roseum, Cunninghamella echinulata, and Fusarium sp., were grown on potato-dextrose broth, 35 cc. of media in 100-cc. bottles, and on potato-dextrose agar in Petri dishes. After 10 days' incubation, the mycelial mats were removed from the broth cultures, and 15 cc. of the culture media placed in sterile Petri dishes containing 100 Illini soybean seeds.

TABLE 5.—The influence of Aspergillus spores and staled culture media on the germination and vigor of Illini soybeans; notes taken 7 days after planting

	Seeds	s soaked in med		ulture		Seeds soa susp	ked in sp ension ^b	ore
	24-h	r. soak	48-h	r. soak	24-h	r. soak	48-h	r. soak
Fungus	Seeds germi- nating	Plants with 1st leaves ex- panded	Seeds germi- nating	Plants with 1st leaves ex- panded	Seeds germi- nating	Plants with 1st leaves ex- panded	Seeds germi- nating	Plants with 1st leaves ex- panded
A. flavus	Pct. 15 26 84 86 82 74	Pct. 0 42 90 87 82 0 85	$egin{array}{c} Pct. & 0 & & & & & & & & & & & & & & & & & $	Pct. 0 5 80 77 84 0 88	Pct. 36 78 77 78 77 50 72	Pct. 0 86 84 85 84 68 83	Pct. 2 64 60 67 72 47 69	Pet. 0 84 83 91 82 55 88

a For the check, seeds were soaked in sterile potato-dextrose broth. b For the check, seeds were soaked in sterile distilled water.

The seeds remained in the liquid for 24- and 48-hour periods, after which duplicate rows, 100 seeds to the row, were planted in sand in a greenhouse at 70° F. Petri-dish cultures were washed with 35 cc. sterile water, and 15 cc. of the spore suspension so obtained was placed in sterile Petri dishes containing 100 Illini soybeans, the beans being left in this spore suspension for 24 or 48 hours before planting. Seeds soaked in sterile broth served as controls for the culture-media test and seeds soaked in sterile water furnished the controls for the spore suspensions.

TABLE 6.—The influence of spores and staled culture media of Aspergillus flavus and A. niger on the viability and vigor of Illini soybeans

	14	Aspergil	llus flavu	8	Aspergillus niger					
	Seeds not treated			treated Arasan		ds not eated	Seeds treated with Arasan			
Treatment	Seeds germi- nating	Plants with first leaves ex- panded	Seeds germi- nating	Plants with first leaves ex- panded	Seeds germi- nating	Plants with first leaves ex- panded	Seeds germi- nating	Plants with first leaves ex- panded		
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.		
Dusted with spores	91	61	96	98	88	89	90	88		
Soaked 12 hr. in spore suspension Soaked 12 hr.	66	36	76	79	64	67	80	73		
in sterile distilled water	65	81	69	80	70	69	75	68		
Soaked 12 hr. in staled culture medium	49	0	56	0	59	71	67	57		
Soaked 12 hr. in sterile potato-dex-	- 0	00		00	70	01	74	50		
trose broth	78 90	83 91	74 96	88 94	72 88	81 91	90	76 89		

Only the culture media in which Aspergillus flavus, A. ochraceus, and A. niger had been grown affected seed germination appreciably, while of the spore suspensions only that from A. flavus affected the stand and vigor of the seedlings, although A. niger reduced the germination (Table 5). A severe stunting of seedlings resulted from treatment of the seed with the staled culture medium on which A. flavus had grown. Treatment of seed with a spore suspension of A. flavus also resulted in stunted seedlings. In addition to the reduced seedling vigor, poorer stands resulted from treatment of seed with spores of A. flavus and with the staled culture medium on which this fungus had grown. Thomas (10), working with filtrates of several fungi, obtained similar results on wheat. He found that strains of

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A. flavus produced in culture media a toxic product that reduced the viability of wheat seed.

A more detailed test was made of the effects of Aspergillus flavus and A. niger. Cultures of the fungi were grown on potato-dextrose broth and agar, and after 5 days the inoculated potato-dextrose broth was passed through filter paper after removal of the mycelial mat. Seeds were soaked in the filtered culture medium and in a spore suspension of the fungi for 12 hours. Although the filtration did not remove all spores from the broth, the number of spores present in comparison to that in the spore suspension was very small. The seeds were then placed between filter paper to remove excess moisture. Other seeds were dusted with spores of the fungi, by

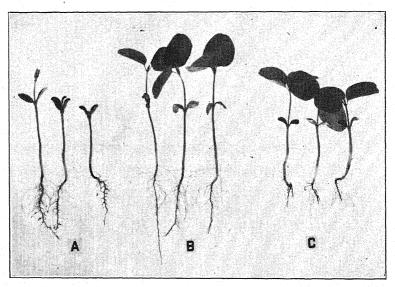


Fig. 3. Effect of Aspergillus flavus and A. niger on seedling development of soybeans. A. From seeds dusted with spores of A. flavus. The cotyledons are large and somewhat swollen while the plumule is greatly retarded in its growth. B. From non-inoculated seeds. C. From seeds soaked for 12 hours in liquid medium on which A. niger had grown. The seedlings are stunted but development of the plumule has not been retarded.

placing 100 seeds on a profusely sporulating culture in a Petri dish. For controls, seeds were soaked in sterile broth and in sterile water. Seeds from each of these lots, after having been dried on filter paper, were treated with the maximum-adhesion dose of Arasan; and duplicate 100-seed rows were planted in sand in a greenhouse at 70° F. immediately after treatment.

The results were similar to those obtained earlier with Aspergillus flavus, but the staled culture medium of A. niger, while reducing germination, did not give such pronounced results as in the previous experiment (Table 6). However, the fungi were grown for only 5 days in broth, and the seeds were soaked in the staled culture medium for 12 hours in contrast with the earlier soakings of 24 and 48 hours. Seeds dusted with spores of A. flavus

gave rise to seedlings that were abnormal. These plants were tall and the percentage germination excellent, but the production of the first leaves lagged behind those of the check, the plants resembling very closely the weak seedlings shown in figure 1. Moreover, there was a tendency for the cotyledons to remain full size, or even increase their size, while at the same time they retained their dark green color. Seedlings of the same age from seeds inoculated with dry spores of A. flavus and then treated with Arasan grew normally, a strong plumule being formed in two weeks, the cotyledons by then beginning to shrivel and turn yellow (Fig. 3).

Only 49 per cent of the seeds germinated after soaking for 12 hours in staled culture medium of A. flavus, and all of the seedlings were stunted. Treatment of the seed with Arasan increased germination slightly, but the seedlings were still dwarfed. Somewhat higher germination (66 per cent) occurred after the seeds were soaked in the spore suspension of A. flavus and 36 per cent of these seedlings were normal. Seed treatment increased the stand to 76 per cent, and 79 per cent of the seedlings were normal. It is evident also that soaking of seeds in sterile water, as Eyster (3) earlier showed with soybeans, or sterile broth for 12 hours prior to planting reduces the stand.

Further tests with Aspergillus flavus, A. niger, and A. ochraceus showed that the spores of A. flavus alone affected the vigor of soybean seedlings, while seeds covered with spores of A. niger and A. ochraceus produced normal seedlings.

DISCUSSION

The results show definitely that microorganisms, especially fungi belonging to the genus Aspergillus, are important in the loss of viability of soybeans in storage and probably are responsible for much of the heating of soybeans up to temperatures of 40-45° C. Seeds with a high moisture content, 13 per cent and up, become moldy when stored at room temperatures, and many of the seeds are infected with Aspergillus spp. High-moisture samples of soybeans, stored under conditions of increasing temperature, also have increasing numbers of seeds internally infected with Aspergillus spp. The number of infected seeds reached a maximum at about 45° C. and diminished at higher temperatures, until at 60° C. no seeds were found infected by fungi or bacteria. The close association of changes in the composition of the seed flora with rising temperature indicates that fungi, especially Aspergillus spp. are responsible for much of the heating. It is probable that the respiratory activities of these microorganisms contribute much to the high respiration rates observed by Ramstad and Geddes (8) in soybeans stored at moderately high temperatures.

Soaking seeds in filtered culture media on which species of fungi isolated from soybean seed had grown demonstrated the sharp specificity of 3 species of Aspergillus (A. flavus, A. niger, and A. ochraceus) in causing abnormal seedling development, while 11 other fungi, including Alternaria and Fu-

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sarium spp., had no effect on seed germination. In addition, seeds dusted with spores of A. flavus gave rise to seedlings in which the plumule of many plants developed very slowly, producing retarded seedlings. However, spores of A. niger or A. ochraceus had no effect on seed germination or on seedling vigor. It is possible that spores of A. flavus developed on moldy soybeans in storage could be spread to uninjured seed, resulting in the development of weak seedlings from such seed. Fungicidal treatment of the seed dusted with spores of A. flavus counteracted the effect of the fungus spores, permitting normal seedlings to develop.

SUMMARY

A survey of the microflora of soybean seeds from Central and Southern Minnesota in 1942 showed that Alternaria spp. were most frequently found, with Fusarium spp. and bacteria next in frequency. The percentage of seeds infected with fungi and bacteria increased in proportion to the frost injury.

The percentage of seeds infected by fungi, especially Aspergillus spp., increased as the moisture content of soybean seeds increased, while the viability and vigor of the high-moisture-content seeds, above about 13 per cent, rapidly decreased.

The percentage of seeds infected with Aspergillus spp. increased as the temperatures under which the seeds were stored increased. The maximum number of infected seeds was found at about 45° C., while at 60° C. no seeds were infected.

Aspergillus flavus, A. ochraceus, and A. glaucus predominated on infected seeds, while A. niger and A. fumigatus were less frequently found. Cephalothecium roseum, Rhizopus nigricans, Cunninghamella echinulata, Chaetomium sp., and Penicillium spp. were also obtained from soybean seed.

Severe retardation in seedling growth resulted from storage conditions favoring development of Aspergillus spp. but fungicidal treatment of the seed improved the vigor and stand of the plants. Spores of A. flavus dusted on soybean seeds resulted in the retarded development of seedlings, while spores of A. niger, A. ochraceus or of any other fungus obtained from soybean seed caused no abnormal seedling development.

UNIVERSITY FARM.

ST. PAUL, MINN.

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RESISTANCE TO SEPTORIA LEAF SPOT AND ITS INHERITANCE IN TOMATOES¹

C. F. ANDRUS AND G. B. REYNARD²

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INTRODUCTION

The leaf spot caused by Septoria lycopersici Speg. has been one of the most injurious foliage diseases of tomatoes (Lycopersicon esculentum Mill.) particularly in eastern and central United States. Under circumstances most favorable for its development, it is capable of causing complete defoliation. An important degree of resistance to Septoria leaf spot, virtually unknown previous to 1941, has been discovered in a number of wild types of Lycopersicon by several independent observers (1, 4, 5, 6, 9), and breeding work has advanced to the point where control of the disease in commercial varieties seems definitely attainable. The present paper records the results of work at the Southeastern Regional Vegetable Breeding Laboratory on Septoria resistance and its inheritance. The work represents one part of an extensive program for the control of tomato diseases by plant breeding.

METHODS

The culture of Septoria lycopersici used in this work was isolated in 1941 from tomato leaves received from Dr. B. B. Higgins, Experiment, Georgia. The culture sporulates copiously on cooked bean pods. Figure 1 shows the semi-liquid masses of spores exuded from pycnidia on the bean pod.

Inoculum was prepared by macerating the sporulating cultures on cooked bean pods in an electric blender (2) for 2 minutes, then diluting with several volumes of water according to the strength of inoculum desired. Such inoculum contains, in addition to spores, large numbers of mycelial fragments on and imbedded in the cooked bean tissues. The strength of the inoculum had no effect on the character of the individual lesions; a very strong inoculum, however, produced multiple lesions whose coalescence caused early death and abscission of the leaves even on plants with the highest degree of resistance. Generally the desirable strength of inoculum for test purposes was that which produced lesions comparable in abundance to the infected leaves shown in figure 2.

Inoculation was accomplished by dipping the foliage of potted plants momentarily into a vessel of inoculum and then keeping them for 48 hours in glass-covered infection chambers in the greenhouse. The plants usually were 28 days from date of seeding, and ranged in height from 5 to 8 inches. Ordinarily a single series of inoculations involved about 1000 plants, which approximated the capacity of the infection chambers. In the inheritance

Contribution No. 42 from U. S. Vegetable Breeding Laboratory, Charleston, S. C.
 Pathologist and Assistant Geneticist, respectively, Division of Fruit and Vegetable
 Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

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study, samples of each parental type were included in each series of 1000 plants.

Leaf-spot readings were made on the seventh day after inoculation. Plants were graded both on the amount of injury and the character of the individual lesions developed upon them. The amount of injury varied with the temperature, strength of inoculum, and duration of time in the infection chamber; the characteristics of individual lesions, however, remained relatively constant. Later in the inheritance study, character of lesion was used as the sole basis of classification, and for this purpose only two types were recognized, A and B, as illustrated in figure 2.

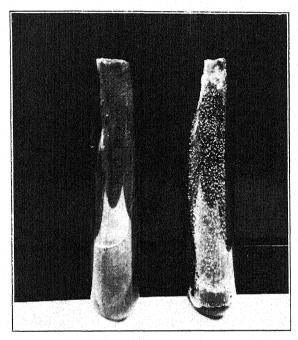


Fig. 1. Culture of Septoria lycopersici on sterilized cooked bean pods. Left, sterile bean pod; Right, richly sporulating growth of Septoria. $\times 1$.

Type A is the familiar Septoria leaf spot, evenly round, diameter 2 to 4 millimeters, with light-colored center usually containing from few to several black dots which are the pycnidia.

Type B lesion is definitely restricted, often pin-point in size and seldom more than 1 millimeter in diameter, dark reddish brown, and containing few or no pycnidia. Occasionally a leaf with type B lesions will also bear a few lesions up to 2 millimeters in diameter with light-colored centers, which are definitely intermediate between types A and B. An intermediate reaction of this type may suggest the behavior of a heterozygous genotype, but in the greenhouse studies the proportion of plants with the intermediate reaction was small and did not seem to correspond with any genotypic class.

In addition to the greenhouse inoculations, there was opportunity in 1942

and 1943 to observe plant reactions in the field following natural dissemination of *Septoria*. All of the inheritance data, however, are based on controlled inoculations in the greenhouse.

RESISTANCE TO SEPTORIA IN COMMERCIAL AND WILD TYPES OF LYCOPERSICON

Several attempts have been made by plant breeders to develop varieties with resistance to Septoria leaf spot and a few commercial types have been introduced which were claimed to possess some degree of resistance to the disease (3, 8). Variety tests in the greenhouse at this laboratory have failed

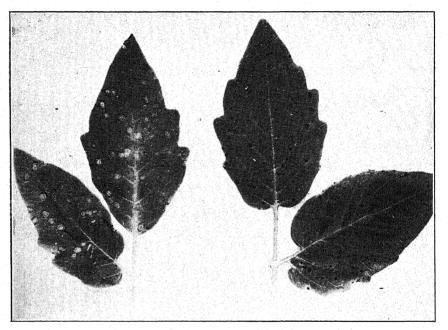


Fig. 2. Septoria lycopersici lesions on tomato leaves. Left, Type A lesions as they appear on susceptible commercial varieties; Right, Type B restricted lesions as they occur on resistant selection M6. $\times 1$.

to demonstrate Septoria resistance in any commercial tomato. All of 127 commercial varieties tested by inoculation were highly susceptible.

In addition to the commercial varieties, this laboratory has tested by inoculation 267 accessions of Lycopersicon received through the Division of Plant Exploration and Introduction of this Bureau. None of these were immune to Septoria, but 12 possessed some degree of resistance, usually represented by rather small lesions intermediate between types A and B. None of the 12 were of the commercial L. esculentum type; 10 of them on the basis of fruit and foliage characteristics were judged to have resulted from outcrossing of L. esculentum with primitive types, either the currant tomato or the cherry tomato; and 2 were known to be of L. hirsutum ancestry. Certain other accessions of L. hirsutum Humb. and Bonpl. and L. peruvianum

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Alexander (1) recorded evidence of resistance to Septoria leaf spot among 488 tomato introductions from the Division of Plant Exploration and Introduction. His data indicate that 67 of the 488 lines possessed some degree of resistance, but only 4 of the 67 approached the commercial types of Lycopersicon esculentum in fruit characters. The majority of the resistant lines belonged to the generally uncultivated species, L. peruvianum, L. glandulosum C. H. Mull., L. hirsutum, L. pimpinellifolium (Jusl.) Mill., and some not easily identified types possibly representing interspecific crosses. He found the highest degree of resistance in L. hirsutum.

Locke (6) also found some lines of Lycopersicon hirsutum to be highly resistant to Septoria, and the resistance appeared to be dominant in crosses with L. esculentum. Several L. esculentum × hirsutum hybrids supplied by Dr. R. E. Lincoln of the Indiana Agricultural Experiment Station have been inoculated at the Vegetable Breeding Laboratory and found to possess a very significant degree of resistance to Septoria. These lines, however, tend to be semi-sterile and have not been used in further breeding work.

The source of Septoria resistance used in heritance studies at the Vegetable Breeding Laboratory was a single plant selection derived from segregating lines of the Australian variety Targinnie Red. This variety apparently had become admixed with natural out-crosses so that many segregants were definitely non-esculentum in type. The Septoria-resistant segregant, called M6, was roughly intermediate between a currant and a cherry tomato in type of fruit and foliage. It proved to be completely fertile in crosses with commercial tomatoes. The Septoria resistance in M6 is equal in degree and apparently identical in type with that found in the best lines of Lycopersicon hirsutum.

The original Septoria-resistant segregant proved to be segregating in respect to resistance to Fusarium wilt (Fusarium oxysporum f. lycopersici (Sacc.) Snyder & Hansen), collar rot (Alternaria solani (Ell. and G. Martin) Jones and Grout), leaf mold (Cladosporium fulvum Cke.), and gray leaf spot (Stemphylium solani Weber) in addition to Septoria leaf spot. By continued selections certain lines were isolated which possessed a high degree of resistance to all five of these important diseases.

Breeding work with the Septoria-resistant tomato has advanced several generations. Original crosses were made in 1942 with the varieties Rutgers and Victor. True backcrosses were made to the original susceptible parents, and in addition the F₁ progenies were out-crossed with the varieties Marglobe, Indiana Marglobe, Gulf State Market, Montgomery, Stokesdale, and Pan America. Much breeding work remains to be done, yet the attainment of an important degree of Septoria resistance in commercial varieties seems probable.

INHERITANCE OF RESISTANCE TO SEPTORIA, IN FIRST, SECOND, AND THIRD FILIAL GENERATIONS

First generation hybrids from resistant \times susceptible crosses were represented by four F_1 plants, all of which were resistant to Septoria.

Second generation hybrids were represented by 10 populations from crosses of M6 (the Septoria-resistant tomato) with the susceptible varieties, Rutgers and Victor (Table 1). Deviations from a 3:1 ratio were significant in 2 samples, which is slightly greater than expectancy. The combined F₂ ratio (709R to 207S) conforms very well with a calculated 3:1 ratio. By elimination of one sample of 29 plants the heterogeneity chi-square is low enough that the remaining populations can be combined satisfactorily. The 15 resistant plants in this separate sample are being grown for further study.

TABLE 1.—Inheritance of Septoria resistance in ten F_2 populations from resistant \times susceptible tomato crosses

		γ^2		
Cross	Total	Resistant	Susceptible	(3:1)
$ ext{M6} imes ext{Rutgers}$	122 256	98 205	24 51	1.847 3.521
${f M6} imes{f Victor}$	29 61 81 22 23 44	15 46 57 17 19 30	14 15 24 5 4 14	8.379** 0.005 0.926 0.061 0.709 1.091
$ ext{M6}13 imes ext{Victor}$	179 99	137 85	42 14	0.225 6.226*
				21.990
	916	709 Heterogene	207 ity χ² (9 D.F.)	$\begin{vmatrix} 2.818 \\ 19.172* \end{vmatrix}$

^{*} Significant at 5 per cent level.
** Significant at 1 per cent level.

Data for the third generation are confined to 26 families representing progeny of 26 plants classified "resistant" in F_2 . According to the single factor hypothesis the F_2 resistant plants should prove to be either segregating or homozygous-resistant in the proportion of 2:1. This expectation was approximated, since of the 26 families, 18 were segregating (heterozygous) and 8 were resistant (homozygous).

The 3:1 type of inheritance is further indicated by the combined ratio within the 18 segregating F_3 populations, none of which deviated significantly from the 3:1 ratio (Table 2).

Classification of the plants was extremely simple; they fell into one of two reaction classes—either with the restricted type of lesion characteristic of the resistant parent (Fig. 2, right) or with the much larger light-centered lesion characteristic of most susceptible varieties (Fig. 2, left). No intermediate grades of plant reaction were distinguishable and dominance of the

TABLE 2.—Inheritance of Septoria resistance in 18 segregating ${\it F}_{\it 3}$ tomato families

Cross	No. of plants			y ²
Cross	Total Resistant		Susceptible	(3:1)
M6 imes Rutgers $M6 imes Victor$	19 19 18 13 18 16 17 16 15 12 16 19 18 15 19 18	17 13 11 9 14 12 10 13 8 9 12 11 14 10 15 13 13 13	2 6 7 4 4 4 7 3 7 3 4 8 4 5 4 5 4 2	2.123 0.439 1.852 0.231 0.074 0.000 2.373 0.333 3.756 0.000 0.000 2.965 0.074 0.556 0.158 0.074 0.020 0.444
	307	224 Heterogene	83 eity χ² (17 D.I	0.678 F.) = 14.791

factor for resistance seemed to be complete. Individual lesions of an intermediate type occurred infrequently on resistant plants, but the reaction of the plant as a whole was clearly either resistant or susceptible.

INHERITANCE OF RESISTANCE TO SEPTORIA IN BACKCROSSES TO THE SUSCEPTIBLE GENOTYPE

According to the single factor hypothesis, with resistance dominant over susceptibility, backcrosses to the susceptible parent should give progenies

 ${\it TABLE~3.--Inheritance~of~resistance~to~Septoria~in~backcrosses~to~susceptible~tomato~varieties}$

	No. of populations			2
Cross	Total	Segregating	Homozygous susceptible	χ^2 $(1:1)$
A. True backcrosses: (M6 × Rutgers) × Rutgers (M6 × Victor) × Victor	10 10	5 2	5 8	0.000 3.600
B. Pseudo-backcrosses: (M6 × Rutgers) × Gulf State '' × Montgomery '' × Pan America (M6 × Victor) × Indiana Marglobe '' × Pan America	8 15 16 6 7	4 9 7 3 3	4 6 9 3 4	0.000 0.600 1.000 0.000 0.143
				5.343
	72	33 Heteroge	39 neity χ² (6 D.F.)	0.500 $= 4.843$

^a To a genotype identical with an original parent with respect to factors under study.

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one-half of which should be susceptible and one-half segregating (heterozygous). This expectation was nearly realized with 20 true backcross populations and 52 pseudo-backcross³ populations (Table 3). None of the deviations from a 1:1 ratio are significant. The backcross of the F₁ to the resis-

TABLE 4.—Inheritance of Septoria resistance in $\it 33$ segregating backcross tomato populations

<u> </u>	No. of plants			γ ²
Cross	Total	Resistant	Susceptible	(3:1)
$(ext{M6} imes ext{Rutgers}) imes ext{Rutgers}$	70 (18) ^a 54 53 36	52 (8) 32 39 29	18 (10) 22 14 7	0.019 7.136** 0.057 0.593
$(M6 \times Victor) \times Victor$	17 19	11 14	6 5	$0.961 \\ 0.018$
Gulf State \times (M6 \times Rutgers)	52 52 (49) ^a 53	42 40 (15) 43	10 12 (34) 10	0.923 0.103 1.063
${\bf Montgomery} \times ({\bf M6} \times {\bf Rutgers})$	61 82 36 20 80 88 54 54 95	43 66 26 17 58 59 38 42 72	18 16 10 3 22 29 16 12 23	0.661 1.317 0.148 1.067 0.267 2.970 0.617 0.222 0.032
(M6×Rutgers) × Pan America	81 75 82 74 (55) ^a 60 79	57 55 61 55 (14) 41 57	24 20 21 19 (41) 19 22	0.926 0.111 0.016 0.018 1.422 0.342
Indiana Marglobe \times (M6 \times Victor)	19 18 19	17 12 14	2 6 5	2.123 0.667 0.018
$(M6 \times Victor) \times Pan America$	18 36 36	13 29 24	5 7 12	$0.074 \\ 0.593 \\ 1.333$
				25.817
	1573	1158 Heterogeneity	415 y χ² (29 D.F.)	1.604) = 24.213

^a Omitted from the final computation.

tant parent offered no advantage from a horticultural standpoint, hence such crosses were not made.

Of the 33 segregating backcross populations, 3 deviated excessively from a 3:1 ratio (Table 4). The segregation in 2 of these populations in fact approximated a 1:3 ratio. The suggestion of reversal of dominance, how-

^{**} Significant at the 1 per cent level.

 $^{^3\,\}mbox{The }F_1$ crossed to a genotype identical with an original parent with respect to factors under study.

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ever, is hardly to be considered in view of evidence for a 3:1 type of inheritance supplied by the great majority of the data in Table 4 and elsewhere. It is believed that the 3 exceptional populations either represent true but exceptional deviations from a 3:1 ratio or are the result of error in handling seed stocks. Differentiation of the two reaction types was again very sharply defined and no misclassification is involved. Resistant plants from the deviating populations were saved for further study.

DISCUSSION

A study of plant disease resistance based on observations made in the greenhouse usually raises the question of the extent to which indoor reactions conform to those that will occur in the field. Generally the agreement between field and greenhouse observations in vegetable plant disease studies has been very close. A few exceptions, however, have been reported and the present instance of resistance to Septoria leaf spot of tomatoes may be another. Locke (6) and the present writers, on the basis of indoor studies in each case, find Septoria resistance to be dominant in inheritance. Wright and Lincoln (9) and Lincoln (5) on the basis of field observations, found such resistance to be recessive. In the latter investigations, however, the authors considered there was some question as to the validity of their hybrid material which involved a cross of L. peruvianum var. humifusum (C. H. Mull.) with L. esculentum.

The apparent disagreement in respect to dominance of Septoria resistance may suggest lack of conformity between indoor and outdoor observations or it may suggest that different genetic factors are involved in the several crosses. On the other hand, neither of these apparent conclusions may be warranted. Our own observations, both in the field and greenhouse, reveal a close general agreement in Septoria reaction. Selected lines that produced the restricted type of lesion indoors produced them likewise in the field, but in addition the older leaves near the base of the stem bore somewhat larger lesions in the field, often with a few pycnidia. This reaction of older leaves tends to escape observation in greenhouse tests where young plants are used exclusively. In the field the presence of any lesions with pycnidia may lead one to classify the plant as fully susceptible, especially since the restricted lesions which distinguish the resistant plant may be obscured by other pathological conditions usually present on field-grown plants.

Considering the nature of the disease and the method of dissemination of the causal organism, it is probable that an intermediate degree of resistance, such as is revealed in these studies, will be greatly increased in value when plants possessing it are grown in solid stands more or less isolated from fully susceptible plants. The near-by susceptible plants are a prolific source of spores which produce many restricted lesions on the resistant plants; coalescence of many such lesions causes a degree of injury that would not occur where the source of reinfection is limited to plants of the resistant type. Thus the circumstances surrounding a field study of disease resistance are apt to result in an unfair evaluation of the resistance potentialities of

a plant, and for this reason alone one should not overlook the value of indoor studies where many factors are under at least some measure of control.

Regardless of the ultimate value of the degree of resistance to Septoria described herein, the restricted lesion characteristic is highly distinct and so far as is known it represents the highest degree of resistance to Septoria available in the genus Lycopersicon. This restricted lesion character is inherited as a single factor and is clearly dominant over full susceptibility. The symbols Se se (Septoria) are suggested to distinguish them from the symbols S s for simple inflorescence and Sp sp for self-topping as listed by MacArthur (7). Little is known concerning the linkages of the factor Se se, but parallel studies now under way indicate that it is independent of the factors for resistance to wilt (Fusarium oxysporum f. lycopersici) and to gray leaf spot (Stemphylium solani).

STIMMARY

All of 127 commercial varieties of tomatoes tested by inoculation were highly susceptible to leaf spot caused by Septoria lycopersici. of resistance to Septoria was found in 12 of 267 accessions of foreign origin. These 12 were known to be of Lycopersicon hirsutum or L. peruvianum ancestry, or their characteristics definitely indicated outcrossing with some wild type.

The highest degree of resistance is characterized by a restricted type of lesion much smaller than normal and bearing few or no pycnidia.

Inheritance studies of crosses of commercial varieties with Septoria-resistant lines were made on 916 F2 and on 3630 F3 and backcross plants involving 70 populations.

Septoria resistance as represented by the restricted type of lesion (symbol Se se) is shown to be inherited as a dominant single factor.

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SOUTHEASTERN REGIONAL VEGETABLE BREEDING LABORATORY, CHARLESTON, S. C.

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INHERITANCE OF RESISTANCE TO THE COLLAR-ROT PHASE OF ALTERNARIA SOLANI ON TOMATO¹

GEORGE B. REYNARD AND C. F. ANDRUS 2 (Accepted for publication July 10, 1944)

INTRODUCTION

In an earlier paper (3) it was shown that resistance to the collar-rot phase of Alternaria solani (Ell. and Mart.) Jones and Grout, was present in several species, varieties, and selected lines of tomato (Lycopersicon spp.). It was also suggested that the control of this disease might be effectively approached through plant breeding. In the process of breeding a commercially desirable, resistant variety, several crosses were made between lines which were either clearly resistant or clearly susceptible to collar rot. Second generation seedlings from these crosses showed segregation for resistance. These facts led to a study of the inheritance of this resistance, both as an aid in determining the most appropriate crossing procedures and as an addition to the knowledge of hereditary factors in the tomato. This paper records the results of the inheritance study.

METHODS

Tomato plants to be used as parents were grown in a screened greenhouse where all original crosses were made. Three types of crosses were made: resistant \times susceptible, resistant \times resistant, and susceptible \times susceptible. In the first type, two sets of reciprocal crosses were analyzed. The F_1 was crossed where possible to one or both parents or to parents apparently identical with the original lines in collar-rot reaction. Second and third generation plants were for the most part field-grown. Occasional individual hybrids from the latter were "off type" for their respective populations, indicating uncontrolled outcrossing in the field, and were discarded for the genetic study.

The classification of all parent lines and hybrids was made on seedlings grown and inoculated as described previously (3), and the same strain of Alternaria used in the earlier work was used here. The varieties Devon Surprize and Bonny Best also were inoculated with each series of plants tested, as resistant and susceptible checks respectively. In addition, uninoculated seedlings of Bonny Best were included as a check for the presence of interfering organisms possibly present in the soil of the inoculation bench.

¹ Contribution No. 41, a report of work performed at the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C., chiefly under an allotment from the Special Research Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935.

² Assistant Geneticist and Pathologist, respectively, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

cultural Research Administration, United States Department of Agriculture.

The authors wish to express their indebtedness to Mr. Hans Jorgensen, assistant scientific aide, and Miss Mary Cele Smith, Jr. scientific aide, for assistance in handling the plants and in compilation of the data involved in this study.

Previous experience in grading collar-rot severity had shown that a wide range of reactions might be expected in segregating lines. The five arbitrary infection classes (Fig. 1) established, the description of each, and the numerical index assigned, were as follows:

$Infection\\ class$	Description	Numerical index
I	Dead	0
II	Bent: seedling alive but broken over at collar lesion	25
III	Intermediate: erect but with well-developed collar lesion	50
IV	Healed: with shallow or definitely healed lesion	75
v	Free: no collar lesion, or only minute points of infection	100

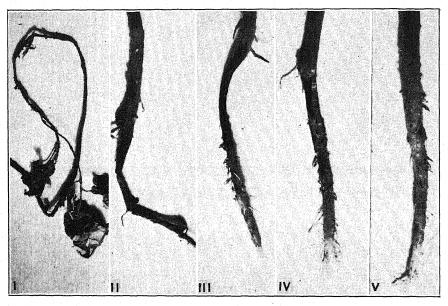


Fig. 1. Tomato seedlings illustrating the five arbitrary classes of collar-rot reaction: I, Dead; II, Bent; III, Intermediate; IV, Healed; and V, Free.

To get the collar-rot rating for any given line, the number of seedlings falling in each class was multiplied by the respective index of that class and the sum of the five products divided by the total number of seedlings inoculated. Thus low ratings indicated collar-rot susceptibility and high ones, collar-rot resistance.

GENERAL RESULTS

Collar rot developed abundantly in each series of inoculations. Devon Surprize maintained its resistance and Bonny Best its susceptibility. There was some difference in the severity of the disease developing in the different series, but the results of the inoculations were taken as recorded without any adjustment of the original method of classification.

In order to establish the grouping of the five infection classes most likely to represent genetic groupings, two sets of inoculations were analyzed. The first included F_1 seedlings and parents from ten crosses of resistant \times sus-

ceptible lines. The results are in table 1. The mean rating of the nine different susceptible parents was 16.4 and of the resistant parents, represented by ten samples from Devon Surprize, was 86.1. The range of ratings observed, from 4.3 to 34.0 in the former and from 79.8 to 98.4 in the latter, illustrates the variations in reaction usually found in separate tests but also shows the sharp differentiation of susceptible and resistant lines. The F_1 ratings were higher than those of the respective susceptible parents in each case and the mean F_1 rating, 37.0, was clearly intermediate between the two mean parental ratings. In crosses 1 and 10, the F_1 ratings were not significantly higher than the ratings of the respective susceptible parents, but

TABLE 1.—Collar-rot reaction of parents and of F_1 progenies in ten crosses, resistant \times susceptible

~		C	ollar-rot ratio	ngs	
Cross No.	Parentage ²	Res.	Susc. parents	$\mathbf{F_{i}}$	L.S.D.d
1.	Devon Surprize × P.I. 126, 452b	81.8	22.7	28.2	7.9
2	Devon Surprize \times F ₄ (V.B.L. $245^{\circ} \times \text{Marglobe}$)	79.8	6.9	18.8	8.4
3 4	Devon Surprize × Montgomery Devon Surprize × Marglobe	88.7 82.2	16.9 17.3	35.6 50.0	8.5 9.2
4 5	Marglobe × Devon Surprize (reciprocal of cross No. 4)	98.4	18.4	42.1	11.9
6	F ₄ (V.B.L. 245 × Marglobe) × Devon Surprize	86.0	8.0	52.3	19.1
7	F ₄ (V.B.L. 245 × Marglobe) × Devon Surprize	93.8	34.0	53.6	10.2
8	Red Cherry × Devon Surprize Red Pear A × Devon Surprize	80.2 89.2	4.3 17.7	25.0 31.4	15.9 10.3
10	Red Pear B×Devon Surprise	81.1	18.1	32.9	17.9
	Mean of ten ratings and its standard error	86.1 ± 2.0	16.4 ± 2.7	37.0 ± 3.8	10.5

^a In these and other crosses, the female parent is listed first.

b P.I. numbers refer to introductions by the Division of Plant Exploration and Introduction of the Bureau of Plant Industry, Soils, and Agricultural Engineering.

Regional Vegetable Breeding Laboratory selection (Red Currant tomato × Marglobe).

d Least significant difference at 5 per cent point between ratings of respective susceptible parents and F₁.

later experience with these crosses did not indicate that they differed genetically from the other crosses.

The intermediate reaction of the seedlings in a heterozygous condition, approaching the reaction of collar-rot-susceptible parents, suggests that susceptibility to collar rot is incompletely dominant over resistance.

The second set of inoculations used in establishing the most probable genetic groupings of collar-rot infection classes involved second and third generation plants. In the spring of 1942, random samples of F₂ seedlings from five crosses of resistant × susceptible lines (Nos. 3, 4, 5, 8, and 10, Table 1) were inoculated. All degrees of resistance and susceptibility appeared. After being graded and separated into the five established classes,

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representatives of the four living classes were transplanted to the field. A total of 371 of these plants lived and reached maturity. Seed was collected from individual plants and the F_3 seedlings inoculated. The results are in table 2.

Of the four collar-rot classes assigned to the F_2 seedlings, represented in the first column of table 2, classes IV and V were the most nearly alike in their proportions of resistant, segregating, and susceptible F_3 families produced. The mean ratings of the two groups of F_3 families, 73.5 and 76.0, did not differ significantly, and it was evident that the F_2 seedlings assigned to these two classes were largely resistant since four-fifths of them (165 in 208) had uniformly resistant progenies.

TABLE 2.—Collar-rot reaction of F_3 families from inoculated and classified F_2 plants from eight crosses, resistant \times susceptible

$\mathbf{F_2}$		$\mathbf{F_3}$	families from	classified F ₂ pl	ants
Collar-rot	No. of		ollar-rot reacti		Collar-rot ratings of all families
classification	seedlings	Uniformly resistant	Segre- gating	Uniformly susceptible	Mean rating and stand- ard error
II, Bent	25 138 99 109	0 15 79 86	14 99 20 23	11 24 0 0	$25.5 \pm 4.32 \\ 38.5 \pm 2.11 \\ 73.5 \pm 2.14 \\ 76.0 \pm 6.95$

In contrast to the similarity of classes IV and V, the reactions of \mathbf{F}_3 families from seedlings classed II and III were significantly different. This is particularly true since all three types of reaction appeared from class III plants and only two from those in class II. The mean ratings of all families from the two groups, 38.5 and 25.5, respectively, also differed significantly. The sharper difference between the ratings of families from classes III and IV gives support to the establishment of a dividing line between these two arbitrarily assigned degrees of collar-rot severity.

The presence of segregating F_3 families from the 208 F_2 seedlings graded "healed" or "free" indicated that they were not all homozygous for collarrot resistance, or, in other words, the grading system was not infallible for every individual seedling. For this reason genetic analysis based on collar rot segregation in the F_2 from crosses of resistant × susceptible lines would not be reliable. It was decided to inoculate both F_2 and F_3 families, but to rely on the latter for critical genetic analysis.

In the first set of inoculations, involving F₁ plants, seedlings of Devon Surprize appeared for the most part in classes IV and V, the two most resistant classes, but some also were found in class III. Repeated progeny tests of Devon Surprize seedlings in class III have invariably indicated resistance equal to that in progeny tests of seedlings put in classes IV and

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V in the same inoculations. This has also been true of other collar-rot-resistant parent varieties. Susceptible parent lines, on the other hand, occasionally gave a small proportion of their seedlings in the resistant classes, IV and V. Progeny tests of these seedlings have indicated susceptibility, suggesting that during the inoculation, occasional seedlings have escaped infection.

For the purpose of the present study, lines that resembled Devon Surprize in collar-rot reaction were considered resistant. In these, the great majority of inoculated seedlings were assigned to classes IV and V with a minority in class III. Lines resembling any of a number of commercial varieties such as Marglobe, Rutgers, or Stokesdale in collar-rot reaction were considered susceptible. Inoculated seedlings of the latter varieties were put largely in classes I and II with some in class III and only very rarely were any in classes IV or V. Lines which consistently gave seedlings in at least four of the collar-rot classes were considered to be segregating.

RESISTANT × SUSCEPTIBLE CROSSES

Fifteen crosses were completed involving three collar-rot-resistant parents (Devon Surprize, Targinnie Red, and a selection from P.I. 127,833) and several collar-rot-susceptible parents. The results of the collar-rot inoculations of the F₂ seedlings from these crosses are in table 3.

TABLE 3.—Collar-rot reaction of F_2 seedlings from 15 crosses, resistant imes susceptible

Cross	Parantaga		No. inoculate seedlings	\mathbf{ed}	
No.	Parentage	Total	Infecti	on class	3:1
		Total	I, II, III	IV, V	
∠ Devon	Surprize \times P.I. 126,452 Surprize \times F ₄ (L. pimpi-	263	208	55	2.343
3 Devon	Surprize × Montgomory	180 174	150	30	6.667*
± Devon	Surprize x Marglaha	695	130 539	$\begin{array}{c} 44 \\ 156 \end{array}$	0.031 2.418
6 Recipro	ocal of cross No. 4	$\begin{array}{c} 465 \\ 148 \end{array}$	350 119	115 29	0.018 2.306
8 Red Ch) × Devon Surprise erry × Devon Surprize ear No. 415 × Devon Sur-	182 478	134 356	48 122	0.183 0.070
10 Red Pe	ear No. 414 × Devon Sur-	248	182	66	0.344
11 Prize	ie Red × Montgomery	460 337	335 241	125 96	1.159
13 Recipro	Surprize × Victor cal of cross No. 12	51 46	36	15	$2.185 \\ 0.529$
vetomo.	ld × Devon Surprize	131	37 105	9 26	0.725 1.855
Litaigion	pe×P.I. 127,835	221	154	67	3.332
					24.165
		4,079	3,076 Heterogene	1,003	0.367

^{*} Significant beyond the 5 per cent point.

In each cross seedlings appeared in at least four of the collar-rot classes, and in most crosses in all five classes, indicating unquestionable segregation for resistance. The numbers of seedlings classified "IV, V," were found to be roughly one-fourth of the total number inoculated. This is also indicated by the relatively low chi-square values obtained for a 3:1 ratio, only one exceeding the 3.841 required for a significant deviation at the 5 per cent point. The excess number of seedlings in this exceptional cross, No. 2, occurred in the more susceptible classes. This deviation in one out of 15 crosses is not considered unusual in view of the experience regarding occasional misclassification of individual seedlings. The heterogeneity chisquare value, barely significant at the 5 per cent point, was reduced to non-significance by omitting this cross, and the accumulated chi-square value of 0.367 indicated a very close fit to the assumed ratio.

From five of the crosses, F₂ populations were grown to maturity and seed saved from individual plants at random. The collar-rot reactions of 251 families from the five crosses are in table 4. A total of 5,496 seedlings was classified in determining the reaction of the 251 families. Variations were

TABLE 4.—Collar-rot reaction of 251 $F_{\rm s}$ families taken at random from five crosses, resistant \times susceptible

Cross			No. of F	3 families		2
No.	Parentage	Total	Suscep- tible	Segre- gating	Resis- tant	1:2:1
4 5 10 14 15	Devon Surprize × Marglobe	47 65 46 47 46	12 10 15 15 6	26 38 21 22 27	9 17 10 10 13	0.915 3.369 1.391 1.255 3.478
						10.408
		251	58	134	59	1.159
			He	terogenei	ty χ²	9.249

found in the proportions of the three classifications but none deviated significantly from a 1:2:1 ratio as was shown by the chi-square values, none of which exceeded 5.991. The non-significant heterogeneity chi-square value indicated that the segregation was essentially the same in each of the five crosses.

RESISTANT × RESISTANT CROSSES

Four crosses were made involving parents classified as collar-rot resistant. The results of the inoculations of these crosses in the first and second generations and of two of the crosses in the third generation are in section A of table 5. All seedlings in the first and second generations of all crosses were resistant. In crosses No. 41 and 43, a total of 78 F_3 families were uniformly resistant with the exception of one segregating family in cross No. 43. All ratings were over 50, the lowest being 59 for the F_1 in cross No. 42.

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In this and related studies, segregating populations have occasionally appeared in lines thought to be uniformly resistant. In some instances this has been traced to field crossing. Although no supporting data are at hand, the presence of one segregating F_3 family in a resistant \times resistant cross (cross No. 43, Table 5) is thought to be due to chance pollination of a resistant F_2 plant in the field by one of several collar-rot-susceptible plants growing in the field at the same time. The possibility of other factors affecting collar-rot resistance is not, of course, excluded.

SUSCEPTIBLE × SUSCEPTIBLE CROSSES

Seven crosses were made involving parents classified as collar-rot susceptible. The results of the inoculations of these are in section B of table 5. All parents and first and second generation populations were susceptible. In contrast to the reactions of the previous group of crosses, all ratings were below 50, the highest being 31 in the F_2 of cross No. 46. Some of the variation in the ratings of individual lines is due to the fact that several separate inoculation series are represented in the table. In all cases, however, the parents and hybrids of any one cross were included in a single inoculation series.

TABLE 5.—Collar-rot reaction in four resistant \times resistant crosses and in seven susceptible \times susceptible crosses

Cross	Collar-rot reac	tions and ratings	s (in italics) a	
No.	Parentage	\mathbf{F}_{1}	$\mathbf{F_2}$	F ₃ families
	A. Resistant × resistant			
41	P.I. 127,814 × Dev. Surprize	resistant	resistant	33 families resistant
42	Dev. Surprize × Red Cherry 88 96	${{\rm resistant}\atop 59}$	resistant 86	
43	Dev. Surprize × P.I. 126,923	resistant	resistant* 91	44 families resistant, 1
44	Norduke × Dev. Surprize	resistant <i>85</i>	resistant 91	segregating
	$\begin{array}{c} \text{B. } \textit{Susceptible} \times \textit{susceptible} \\ \textit{crosses} \end{array}$			
45	P.I. 128,602 × P.I. 95,588	susceptible	susceptible	
46	P.I. 126,452 × Marglobe	susceptible	susceptible	
47	Marglobe × Montgomery		susceptible	
48	Montgomery × Marglobe5	susceptible	susceptible 5	
49	Red Pear × Cooper Special	susceptible	susceptible	
50	P.I. 118,324 × Montgomery	susceptible	susceptible	
51	P.I. 95,588 × Marglobe	susceptible	susceptible	

² Figures under variety names or progeny groups represent numerical reactions ratings.

In making the collar-rot classifications listed in table 5, 229 F_1 , 1402 F_2 , and 1838 F_3 seedlings were inoculated, in addition to approximately 400 seedlings from the parents of the crosses.

BACKCROSSES

Three backcrosses, (resistant \times susceptible) \times susceptible, were tested. In addition F_1 plants were outcrossed to other collar-rot-susceptible parents

TABLE 6.—Collar-rot reaction in second generation populations of backcrosses. A, (resistant × susceptible) × susceptible; B, (resistant × susceptible) × resistant

Cross	B	Num	ber of sec popul	ond gene ations	ration	χ² 1: 1
No.	${ m Parentage^a}$	Total	Sus- ceptible	Segre- gating	Resist- tant	1:1
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31	A. $F_1 \times susceptible\ parent$ Cross No. $12 \times Rutgers$ "" $\times Valiant$ "No. $13 \times Rutgers$ "" $\times Valiant$ ""	21 14 19 15 17 18 4 14 3 13 14 2 2 3 13 4	9 7 11 8 5 6 1 4 0 7 6 1 2 4 2 2	12 7 8 7 12 12 3 10 3 6 8 1 1 9	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0.428 0.000 0.474 0.667 2.882 2.000 1.000 2.571 3.000 0.769 0.286 0.000 0.333 1.923 0.000 4.454*
32 33 34 35 36	(Dobbies Champion × Victor) × Rutgers '' × Montgomery '' × Stokesdale '' × Pan America '' × Victor	9 2 18 24 16	5 0 8 14 8	4 2 10 10 8	0 0 0 0	0.909 2.000 0.222 0.667 0.000
		254	110	144 Heterog	0 encity χ^2	0.455 $= 24.130$
37 38	B. $F_1 imes resistant\ parent$ Cross No. $4 imes Danish\ Export$ '' $ imes Devon\ Surprize$	35 8	0	16 4	19 4	$0.257 \\ 0.000 \\ \hline 0.257$
		43	0	20	23	0.209
			-	Hetero	geneity ;	$z^2 = 0.048$

^a Crosses 4, 5, 12, and 13 are listed in table 3. * Significant beyond the 5 per cent point.

similar in reaction to the original parents, and these crosses are treated as backcrosses. Seed was saved from individual first generation plants of the backcrosses and second generation seedlings were inoculated. The results of the inoculations are in section A of table 6. The direct backcrosses are

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Nos. 20, 22, and 36. A total of 4,802 seedlings was inoculated in classifying the 297 families.

On the assumption that a single pair of factors is involved in collar rot resistance as is suggested by the analysis of second and third generation populations, half of the populations from each backcross would be expected to be susceptible and half segregating. The chi-square determinations were made accordingly for a 1:1 ratio. As shown by the data in section A of table 6, in each of the 21 crosses only the two types of reaction occurred, none of the populations being uniform for resistance. One of the chi-square values exceeded 3.841, indicating a deviation from the assumed ratio significant at the 5 per cent point; but the chi-square value of the accumulated segregation, 0.455, showed that the ratio closely approached 1:1.

One backcross, No. 38 (Table 6, B), was made to the collar-rot-resistant parent, Devon Surprize. In addition, in cross No. 37, the F₁ was outcrossed to the variety, Danish Export. This variety had given collar-rot reactions as resistant as Devon Surprize. A 1:1 ratio of resistant to segregating populations was expected and a close approximation, 20:23, observed. None of the 43 populations was uniformly susceptible.

SEGREGATION WITHIN F3 FAMILIES

As a further test of the suggested single factor hypothesis, analysis was made of the seedling segregation within 258 F₃ families. The families selected were those in which segregation was unquestionable, or those in which seedlings usually appeared in five of the collar-rot infection classes (Fig. 1). The results of the analysis, assembled from eleven separate inoculations, are in table 7. Each inoculation included families from one or more of the resistant × susceptible crosses listed in table 3.

In one of the eleven inoculations, No. 81, the chi-square value indicated segregation deviating from a 3:1 ratio; the other ten and the accumulated segregation closely approached this ratio. In the deviating inoculation, an excess of seedlings appeared in the more resistant classes, IV and V, suggesting that a relatively weak infection occurred. The tests of the F₃ seedlings as a whole, however, appear to confirm the suggested type of inheritance.

SEGREGATION WITHIN SECOND GENERATION FAMILIES OF BACKCROSSES

A summary was made of the seedling distribution in 102 clearly segregating second generation populations from (resistant × susceptible) × susceptible. There were 1,549 seedlings classified, and of these 1,182 were in classes I, II, and III and 367 in classes IV and V. This result gives a satisfactory fit to a 3:1 ratio, the chi-square value being 1.343.

DISCUSSION

It seems evident that resistance to the collar rot phase of Alternaria solani behaves as an inherited character and that a single pair of factors

controls this resistance. Several minor deviations represented by significantly high chi-square values for 3:1 or 1:2:1 ratios appeared in the observed segregations. In each case, however, the accumulated chi-square values have been low and in most cases heterogeneity chi-square values indicated similar reactions among a group of different crosses.

There was no indication that the resistance of Devon Surprize, which was used in the majority of crosses studied, differed in degree or type from that of other resistant parent lines or from homozygous resistant hybrids derived from crosses made. It is significant that this variety from Scotland, Targinnie Red from Australia, and P.I. 127,833 from Peru had apparently identical forms of resistance, both visually and from a genetic standpoint.

TABLE 7.—Collar-rot classification in 258 segregating F_2 families from resistant \times susceptible crosses, assembled from eleven inoculations

	dlings	No. of inoculated seedlings			
3:1	on class	Infection	No. of families		Inoculation No.
	IV, V	I, II, III	Total		
0.082	104	302	406	20	11
0.794	37	131	168	9	71
0.653	184	515	699	50	74
0.941	153	502	655	25	75
3.084	284	754	1,038	78	76
0.278	115	365	480	20	77
0.259	76	213	289	12	78
1.246	49	176	225	. 8	80
4.123*	160	397	557	19	81
2.876	25	109	134	5	82
1.526	84	215	299	12	83
15.862					
0.363	1,271	3,679	4,950	258	
$y \chi^2 = 15.499$	Heterogeneity				

^{*} Significant beyond the 5 per cent point.

The great majority of inherited characters that have been studied in Lycopersicon have been reported to be controlled by single pairs of factors (9). Definite evidence of genetic resistance to disease in tomato has been found for such diseases as Fusarium wilt (4, 10), Stemphylium leaf spot (2), Septoria leaf spot (8), Cladosporium leaf mold (1, 5, 6, 11), and possibly western yellow blight (7). In the majority of these cases, resistance has been reported as almost completely dominant or completely recessive to susceptibility. In the present study seedlings heterozygous for collar-rot resistance were usually distinguishable from those homozygous for resistance or susceptibility and their collar-rot reactions more nearly resembled the reaction of susceptible lines. For these reasons, it is considered here that collar-rot susceptibility is only partially dominant over resistance.

In the case of Cladosporium fulvum Cke., Langford (6) found several types of resistance, assigned symbols to the factors involved, and suggested

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the system of symbols employed as a model for subsequent reports defining resistance to other diseases in tomato. In his system, the initials of the Latin name of the disease were used to represent the genes for resistance and subscript letters represented the initial letters of the tomato variety in which resistance was first discovered. The factor for resistance to *C. fulvum* found in the variety Stirling Castle was given as "Cf_{sc}."

Although collar-rot resistance was found in several varieties at the same time (3), the variety Devon Surprize has been used most extensively in this study and will be designated as the typical resistant variety. The symbols suggested are "A_d-a_d," the factor pair governing resistance to the collar-rot phase of *Alternaria solani*, as in the tomato variety, Devon Surprize.

Linkage relationships have not as yet been determined, but one contributing fact was observed; Devon Surprize has even-ripening fruit, the genes for which are located on chromosome No. VII of MacArthur's (9) chromosome map of tomato. This character was inherited independently of collar-rot resistance, indicating that the genes for the latter are probably not located on chromosome VII.

SUMMARY

Tomato lines uniformly resistant and lines uniformly susceptible to the collar-rot phase of $Alternaria\ solani$ were crossed and succeeding generations analyzed for resistance or susceptibility to the disease. Analysis was made of the collar-rot reaction of approximately 500 F_1 , 5,400 F_2 , 7,300 F_3 seedlings and, in addition, 4,800 seedlings from backcrosses. A total of 15 F_2 populations, 251 F_3 families, and 23 backcross populations involving crosses of resistant \times susceptible parent lines was tested. Plants were artificially inoculated in the seedling stage, grown in soil in greenhouse benches and classified according to the severity of the development of collar rot.

Unquestioned segregation for resistance occurred in the hybrids and analysis of second and third generation and backcross populations indicated a simple form of inheritance involving one pair of factors. An intermediate type of reaction was observed in which susceptibility to collar rot appeared to be only partially dominant over resistance. Resistance manifested in plants of the second and later generations appeared to be equal to that found in parent lines. Resistance found in tomatoes from widely scattered sources appeared to be inherited in identical manner.

The forcing variety, Devon Surprize, was used as the principal collar-rot-resistant parent. Following a modification of the system of symbols described by Langford, the factor pair governing resistance to the collar-rot phase of $Alternaria\ solani$ was assigned the symbols " A_d - a_d ."

U. S. VEGETABLE BREEDING LABORATORY, CHARLESTON, SOUTH CAROLINA.

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MOSAIC, STREAK, AND YELLOWS OF CARNATION¹

LEON K. JONES

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INTRODUCTION

The literature dealing with viroses of carnation was recently reviewed by Creager2 and the name "mosaic" was substituted for the name "yellows" for the disease originally described by Peltier.3 Although Peltier3 and Lamkey4 did not definitely say that the disease was caused by a virus, they did demonstrate the infectious nature of the disease by grafting and showed that it was not caused by fungi or bacteria. Lamkey4 concluded that yellows was infectious but not contagious. He also referred to peach viroses and tobacco mosaic and inferred that carnation yellows was a similar type of trouble. Based on priority of usage the name "yellows" should have been used by Creager2 because his description and photographs of foliage symptoms show that he was working with the disease described by Peltier.3 Lowered quality of flowers from yellows-affected plants was reported by Peltier and by Lamkey, but Creager associated the breaking of the color of flowers with this disease.

Viroses of carnation have been under observation in Washington since 1930 and actively investigated since 1937.5 Early is the investigations it was observed that all commercial varieties of carnations were infected with viroses to a greater or lesser extent and that it was advisable to develop virusfree seedlings in order to study the disease. As the work progressed on virus-free seedlings, it became evident that 2 viruses were associated with the disease known as yellows. One of the viruses, the mosaic virus, was generally present in all commercial varieties. The other virus, the streak virus, varied greatly in prevalence in different greenhouses and varieties. Since commercial varieties are practically 100 per cent affected with the mosaic virus, the streak virus was not observed alone in commercial plantings but was always in combination with the mosaic virus. This combination of viruses is the cause of the disease called yellows. It appears that Creager has substituted the term "mosaic" for "yellows" without separating the two viruses. The leaves and bud listed as normal in figures 1 and 2 of Creager's article show mosaic symptoms, and those listed as affected with mosaic show symptoms of yellows. It is interesting that Lamkey stated that the terms "mottle leaf," "mosaic," or "yellow fleck and streak" might be more appropriate descriptive terms than "yellows."

¹ Published as scientific paper No. 612, College of Agriculture and Agricultural Experiment Stations, State College of Washington.

² Creager, D. B. Carnation mosaic. Phytopath. 33: 823-827. 1943.

3 Peltier, Geo. L. Carnation yellows. Proc. Amer. Carnation Soc. 25: 29-35. 1916.

4 Lamkey, E. M. R. A consideration of yellows. Proc. Amer. Carnation Soc. 26:

<sup>25-35. 1917.

&</sup>lt;sup>5</sup> Wash. Agr. Exp. Stat. Ann. Repts. 48: 65-66. 1938; 49: 61-62. 1939; 50: 76-77. 1940; 51: 85. 1941; 52: 74. 1942; 53: 67. 1943.

SYMPTOMS

Mosaic

Slight mottling of the leaves with light-green irregular to elongate blotches (Fig. 1, C), which makes the plant a lighter green than normal, is the characteristic symptom of this disease. The mottling is usually more pronounced in the young leaves than in the older ones. Foliage from virus-free plants is smooth and uniformly green in contrast with the mottled foliage of mosaic-affected plants. Mosaic often shows in the flowers of colored varieties as somewhat lighter streaks that parallel the veins of the petals. The mottling of the foliage and the broken color of the flowers caused by mosaic ordinarily are not sufficiently damaging to be of much economic importance.

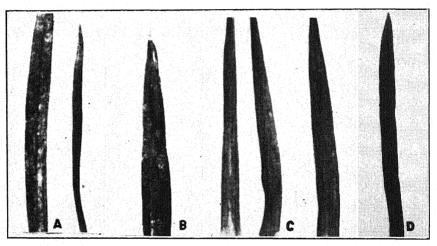


Fig. 1. Mottling and necrosis of carnation leaves caused by viruses. A, yellows; B, streak; C, mosaic; D, virus-free leaf.

Streak

Yellowish or reddish spots and streaks one-half to one mm. wide, either circular or elongate, paralleling the veins are noted on foliage of affected plants (Fig. 1, B). The reddish spots are more pronounced on foliage of colored varieties than the yellowish spots on foliage of light pink or white varieties. On the deep red varieties, such as Potentate, the outer portion of the spot becomes purple in contrast to the light tan center. Many of the lower leaves on affected plants may become severely spotted, turn yellow, and die. The streak virus alone appears to produce no distinguishing symptom on the flower.

Yellows

Since yellows is caused by a combination of the two viruses, mosaic and streak, both the mottling and spotting are characteristic of the trouble (Fig. 1, A). The severity of the foliage mottling and the flower streaking associ-

ated with the mosaic disease are increased by the presence of the streak virus. Severely spotted foliage more often dies with the combination of the 2 viruses than with streak alone. Yellows has been observed to cause the death of nearly all the foliage on flower stems of Virginia and Laddie varieties during March and April. Yellows-affected plants also suffer more severely from Fusarium branch and stem rot than is the case with mosaic-affected plants.

TRANSMISSION

Four series of grafts on 100 virus-free seedlings and 6 series of mechanical inoculations on 272 virus-free seedlings were made from yellows and mosaic plants in 1938–40 before determining that 2 viruses were associated with the yellows disease. Accordingly, the results of these tests were confusing. In October, 1940, grafts from yellows-affected Patrician and mosaic-affected King Cardinal were made on 5 plants, each of a virus-free seedling

TABLE 1.—The transmission of the carnation mosaic and streak viruses

Inoculum	Type of inoculation	Number of trials			Average percentage of plants affected with		
	inoculation	or mais	mocurated	Mosaic	Streak	Yellows	
Mosaic	Mechanicala Grafting (Myzus persicae Thrips tabaci Myzus persicae (((((((((((((6 2 6 3 1 4 2 2 2 1	316 20 86 20 12 147 30 18 92b 5b	99 100 100 0 0 0	0 0 0 100 93 0 0	0 90 90 0 0 0 0 0 94 100	

^a Portion of affected leaf rolled and injured on swab with a pot label before being rubbed on leaf to be inoculated. Leaf to be inoculated was sprinkled with carborundum dust previous to inoculation.

b Plants affected with mosaic before being inoculated in these tests.

clone. Symptoms of yellows developed on plants grafted with yellows-affected scions from Patrician, and mosaic on plants grafted with mosaic-affected scions from King Cardinal. This separation of symptoms was perpetuated in further grafts from affected plants to plants of another seedling clone.

Mechanical inoculations from yellows-affected plants previous to the understanding of the complex nature of the disease indicated that yellows was not transmitted mechanically, but that a mosaic-mottling of the foliage was readily transmitted by mechanical inoculation methods from yellows-affected plants. The use of aphids (*Myzus persicae* Sulzer) gave indications that yellows was transmitted by this insect, because the inoculated plants were carrying the mosaic virus before viruliferous aphids were placed upon them. An understanding of the situation was obtained when aphids were transferred from yellows-affected Patrician to 5 plants of a seedling clone

that had been previously inoculated with mosaic, and typical yellows developed on the dual-inoculated plants.

Since October, 1940, a large number of inoculations by mechanical methods and by the use of insects on virus-free clonal lines of seedlings has shown that the mosaic virus is readily transmitted by mechanical inoculation but is not transmitted by Myzus persicae or Thrips tabaci Lindeman (Table 1). Also, the inoculations have shown that the streak virus is transmitted by M. persicae but not by T. tabaci or by mechanical inoculation methods (Table 1). Preliminary tests show that the mosaic virus is inactivated in

TABLE 2.—Observed reaction of carnation varieties infected with yellows in commercial greenhouses^a

Severe symptoms	Moderate symptoms	Slight symptoms
Betty Lou Bonanza Boston Ward (3) Camelia Chief Kokomo Dimity Ditchling Doris Allwood Fire Chief Gardenia Giant Laddie Golden Wonder King Cardinal Martha Ellen Melrose Patrician Pink Treasure Potentate Puritan (2) Purity (3) Robert Allwood (2) Rose Pink Joan Marie Scarlet Monarch (2) Senator Snow White Tangerine Triumph White My Love (2) Woburn (2)	Achievement Dark Scarlet Del Ray Eleanor Ethel Ivory White Joan Marie Kathryn Maytime Mrs. C. B. Johnson My Love Paragon Peter Fisher Pink Spectrum Red Matchless (1) Red Triumph (2, 3) Rosalie Salmon Spectrum (2) Satellite (1, 2) Spectrum Supreme Topsy (3) Virginia Virginia Rose	Admiration Carlotta Dairy Maid Dictator John Briry Matchless Mrs. Sims Olivette Pelargonium Pharach Pink Abundance Portland Pink Vivel Field Clark (2) Vivian (1, 2)

^a Observations made in 1938-1944 in greenhouses in Washington and (1) Charles City, Iowa; (2) Milwaukee, Wisconsin; and (3) Denver, Colorado.

vitro near 60° C., and remains active in vitro for 7 days but is not active after 42 days. The minimum incubation period of the mosaic virus was 22 days and of the streak virus 25 days, but longer periods of incubation, up to 50 to 60 days, were commonly observed depending upon the vigor of the inoculated plants.

VARIETAL RESISTANCE

Carnation varieties in commercial greenhouses differ in severity of symptoms of yellows shown by affected plants. Observations in Washington, Iowa, Wisconsin, and Colorado recorded in table 2 show the degree of injury

noted on common varieties. Some of the varieties listed as slightly or moderately injured by the presence of yellows in the plant have not been observed under sufficiently varying conditions to be certain of their proper classification.

TABLE 3.—Percentage of plants of carnation varieties showing symptoms of yellows in commercial greenhouses of Washingtona

Variety	Greenhouses	Range	Average
	No.	Per cent	Per cent
Pollyanna	1	0	
Matchless	3	0-7	2
John Briry	6	1-10	4
Dark Pink Maytime	i	10	_
Eleanor	ī	10	
Dorothy Napier	$\frac{1}{2}$	10-20	
Hercules Virginia	2	10-20	15
Pink Treasure	1 1		15
U. S. D. A. No. 1	1	20	*********
Olivette		20	
Pharaoh	4	0-40	25
	2	10-60	35
Maytime	3	2–70	41
Snow White	2	10-75	46
Peter Fisher	5	6-90	47
Joan Marie	7	20-80	54
Com Knipe	3	40-70	50
Puritan	4	10–90	60
Pink Spectrum	4	30–90	61
ardenia	1	67	********
Virginia Rose	6	30-95	69
Pink Abundance	1 1	70	
My Love	5	60-100	78
Melrose	1	80	
Purity	1 1	80	
Rose Pink My Love	1	80	
King Cardinal	10	15-100	81
Chief Kokomo	2	75-90	83
Virginia	$\overline{7}$	60-100	86
Camelia	i	90	
Termosa	i	90	***
Mrs. C. B. Johnson	ī	90	•••••
Ethel	2	90–98	94
Incetuum Cumromo	4	90-100	94
Spectrum Supreme	2	100	
Setty Lou	$\frac{2}{2}$	100	100 100
Fire Chief			100
diant Laddie	1	100	
Paragon	1	100	100
Patrician	2	100	100
Friumph	1 1	100	

^a Observations made in greenhouses in March and April, 1941-1944. The highest percentage observed in a given variety is recorded.

Many of the varieties listed as showing severe symptoms have had sufficient loss of crop to lead to their discontinuance by some growers in Washington during the past 6 years. Most of the varieties listed in table 2 have been developed in the last 15 years. Matchless and Boston Ward appear to be the only varieties observed that have been in production for 30 years. This leads one to conjecture as to the part played by yellows in the "running-out" of commercial carnation varieties in the past. Peltier, in 1916,

that had been previously inoculated with mosaic, and typical yellows developed on the dual-inoculated plants.

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TABLE 2.—Observed reaction of carnation varieties infected with yellows in commercial greenhouses^a

Severe symptoms	Moderate symptoms	Slight symptoms
Betty Lou Bonanza Boston Ward (3) Camelia Chief Kokomo Dimity Ditchling Doris Allwood Fire Chief Gardenia Giant Laddie Golden Wonder King Cardinal Martha Ellen Melrose Patrician Pink Treasure Potentate Puritan (2) Purity (3) Robert Allwood (2) Rose Pink Joan Marie Scarlet Monarch (2) Senator Snow White Tangerine Triumph White My Love (2) Woburn (2)	Achievement Dark Scarlet Del Ray Eleanor Ethel Ivory White Joan Marie Kathryn Maytime Mrs. C. B. Johnson My Love Paragon Peter Fisher Pink Spectrum Red Matchless (1) Red Triumph (2, 3) Rosalie Salmon Spectrum (2) Satellite (1, 2) Spectrum Supreme Topsy (3) Virginia Virginia Rose	Admiration Carlotta Dairy Maid Dietator John Briry Matchless Mrs. Sims Olivette Pelargonium Pharaoh Pink Abundance Portland Pink Vivel Field Clark (2) Vivian (1, 2)

a Observations made in 1938-1944 in greenhouses in Washington and (1) Charles City, Iowa; (2) Milwaukee, Wisconsin; and (3) Denver, Colorado.

vitro near 60° C., and remains active in vitro for 7 days but is not active after 42 days. The minimum incubation period of the mosaic virus was 22 days and of the streak virus 25 days, but longer periods of incubation, up to 50 to 60 days, were commonly observed depending upon the vigor of the inoculated plants.

VARIETAL RESISTANCE

Carnation varieties in commercial greenhouses differ in severity of symptoms of yellows shown by affected plants. Observations in Washington, Iowa, Wisconsin, and Colorado recorded in table 2 show the degree of injury

noted on common varieties. Some of the varieties listed as slightly or moderately injured by the presence of yellows in the plant have not been observed under sufficiently varying conditions to be certain of their proper classification.

TABLE 3.—Percentage of plants of carnation varieties showing symptoms of yellows in commercial greenhouses of Washington²

Variety	Greenhouses	Range	Average
	No.	Per cent	Per cent
Pollyanna	1	0	
Matchless	3	0-7	2
John Briry	6	1-10	4
Dark Pink Maytime	ľ	10	_
Eleanor	ī	10	*********
Dorothy Napier	2	10-20	15
Hercules Virginia	2	10-20	15
Pink Treasure	ī	20	1
U. S. D. A. No. 1	i	20	
Olivette	4	0-40	25
Pharaoh	2	10-60	35
Maytime	3		1
Snow White	2	$\begin{array}{c} 2-70 \\ 10-75 \end{array}$	41
Dotor Dishor			46
Peter Fisher	5	6-90	47
Joan Marie	7	20-80	54
Tom Knipe	3	40-70	50
Puritan	4	10-90	60
Pink Spectrum	4	30–90	61
Gardenia	1	67	
Virginia Rose	6	30-95	69
Pink Abundance	1	70	
My Love	5	60–100	78
Melrose	1 1	80	
Purity	1	80	
Rose Pink My Love	1	80	
King Cardinal	10	15-100	81
Chief Kokomo	2	75-90	83
Virginia	7	60-100	86
Camelia	i	90	
Hermosa	ī	90	
Mrs. C. B. Johnson	ī	90	********
Ethel	2	90-98	94
	4	90–100	94
Spectrum Supreme	2	100	100
Betty Lou	2	100	100
Fire Chief	1 1	100	
Giant Laddie		100	•
Paragon	$\frac{1}{2}$		100
Patrician	2	100	100
Triumph	1	100	

^a Observations made in greenhouses in March and April, 1941-1944. The highest percentage observed in a given variety is recorded.

Many of the varieties listed as showing severe symptoms have had sufficient loss of crop to lead to their discontinuance by some growers in Washington during the past 6 years. Most of the varieties listed in table 2 have been developed in the last 15 years. Matchless and Boston Ward appear to be the only varieties observed that have been in production for 30 years. This leads one to conjecture as to the part played by yellows in the "running-out" of commercial carnation varieties in the past. Peltier, in 1916,

stated that all varieties grown at that time had yellows and that the disease was the most serious trouble of carnations. Comments by a grower in 1916⁶ that he had seen yellows as long as he had grown carnations would lead to the conclusion that it has been an important disease of carnations for a long time.

Yellows was prevalent on many carnation varieties in commercial greenhouses of Washington, in March and April of 1941–1944 (Table 3). The standard varieties Betty Lou, Camelia, Chief Kokomo, Gardenia, Giant Laddie, Mèlrose, Patrician, Puritan, and Purity have been discarded during

TABLE 4.—Observations on yellows in rooted cuttings of carnations purchased by growers in Washington, 1940–1944a

Source and variety of cuttings	Percentage of plants with symptoms of yellows		
Massachusetts Bauerlein's White Charm Dark Pink Maytime	0 0		
New York Olivette	0		
Pink Spectrum Rosalie Snow White	0		
Virginia RoseIndiana	ů .		
Dorothy Napier Maytime Olivette	10, 20 20		
Pharaoh Pollyana Tom Knipe	$\frac{1}{2}$		
VivianColorado	20 10		
Derigo Light Pink Maytime Pink Spectrum	20 50 58		
Spectrum Supreme	56 56		
Oregon Melrose	50		

^a Data collected on young potted plants in greenhouses in Washington within 30 days after the plants had arrived from shipping points.

this 4-year period mainly because of reduced quality and production caused by the yellows disease. Some growers have also expressed the opinion that reduced quality and production makes it desirable to find new varieties to replace Fire Chief, Joan Marie, King Cardinal, My Love, Virginia, and Virginia Rose. High percentages of plants of these varieties were affected with yellows in the different greenhouses (Table 3). The John Briry and Matchless varieties consistently had none to low percentages of yellows. The fact that aphids are seldom found on these varieties except in heavily infested greenhouses no doubt accounts for their relative freedom from the disease.

⁶ See footnote 3.

On the other hand, King Cardinal, Fire Chief, and some other varieties to a lesser extent are very susceptible to aphid infestation.

As the varieties become less productive, growers buy new stock from other parts of the country in order to obtain more productive old varieties or to try new varieties. Many lots of rooted cuttings brought into the State of Washington during the past 5 years have been affected with yellows (Table 4). No definite information was obtained as to the location of the greenhouse in which the cuttings were made but the state from which the cuttings were shipped is recorded in table 4. From these few data it appears that yellows is more prevalent in carnation stock produced in the central and western part of the United States than in stock produced in the northeastern part of the United States.

Seedling carnation plants were developed from seed obtained from England and France in 1938-1939, but crosses were made with commercial varieties in the greenhouse in Pullman in 1938-1940 for the production of A total of 1038 seedlings have been grown with no indication that the mosaic or streak viruses were transmitted to the plants through the seed. Twenty-eight of the most promising seedlings were selected and developed into clonal lines. Inoculation by grafting on these clones has failed to show any immunity to infection by the streak and mosaic viruses, but considerable difference in degree of injury to affected plants has been observed. Two of the clones, that show relatively mild symptoms when affected with yellows have been distributed in lots of 100 and 1200 plants each to 7 growers in the Spokane area for observations under commercial conditions. The 2 varieties are dark rose pink and named Victoria and Genevieve. Victoria came from a cross of Joan Marie × King Cardinal and Genevieve from a cross of King Cardinal x an unnamed red seedling developed from seed purchased in England. Further crosses have been made but the war emergency has curtailed investigations on this project.

THE EFFECT OF NUTRITION ON SYMPTOMS

It was recognized by Peltier that symptoms of yellows were influenced by environmental conditions. Symptoms are often very marked on rooted cuttings in the sand or soon after they are potted. This is especially true if the cuttings remain in the sand beyond an optimal period for root development. The use of hormones to assist in rooting cuttings has been considered by growers to be responsible for the development of severe symptoms. Tests with hormones at Pullman indicate that symptoms are no more severe on treated cuttings than on untreated cuttings. There is the possibility that the more rapid rooting and greater abundance of roots on affected treated cuttings would make them suffer more severely from a depleted nutrient condition than untreated cuttings, if left in the sand too long before potting. Cuttings from yellows-affected plants do not strike root easily and lower percentages of rooted plants are obtained from them than from virus-free or mosaic-affected plants.

Observations and discussions with growers would lead to the conclusion that unfavorable growing conditions that injure the root system or check plant growth increase the development of symptoms in diseased plants. Low fertility of soil, excessive amounts of salts in the soil, too heavy and frequent applications of water, and excessive applications of fertilizer are factors that appear to have been responsible for severe symptom development in various greenhouses under observation. In the fall and winter the symptoms of yellows are usually mild unless aggravated by very unfavorable soil conditions. In the spring, however, when heavy growth is made with heavy watering and no fertilizing the symptoms may become very severe. Injury to the root system in potting from the sand, repotting, or transplanting has been observed as a possible factor in increasing symptoms.

Yellows-affected Patrician plants were placed in neutral peat in glass pots October 6, 1938, and grown through the winter with the addition of nutrient solutions as the moisture condition of the peat showed the need.

TABLE 5.—The effect of nutrients upon the development of carnation yellows $symptoms^a$

Nutrient solutions	No. plants	Degree of symptoms on plants			Av. number spots per	
	grown	Medium	Severe	Dead	shoot	
Complete nutrient Complete minus nitrogen Complete minus phosphorus Complete minus potassium	12 12 12 12 12	5 3 3 4	0 6 8 8	0 3 1 0	8 47 92 74	

a Patrician plants grown in pots of neutral peat.

Twelve pots each were treated with complete nutrient, complete nutrient minus phosphorus, and complete nutrient minus potassium solutions. Severe symptoms appeared on the plants in the pots receiving complete nutrient minus phosphorus, and complete nutrient minus potassium solutions on October 18, 1938, but plants in the other solutions were free of severe symptoms. The final data taken February 6, 1939 (Table 5), show that the lack of nitrogen, phosphorus, or potassium from the complete nutrient solution gave increased symptoms on plants and that the plants were more severely damaged from a lack of potassium or phosphorus than from a lack of nitrogen.

The soil in 27 benches of carnations in 15 greenhouses was analyzed in March, 1942, according to the technique described by Spurway.⁸ Tests were made for nitrates, potassium, phosphorus, chlorides, and soil reaction. Total salts in the soil of each bench were determined with the "Washington State Soil Tester" developed by E. C. McCulloch and L. C. Wheeting. The carnation plants in 12 of the benches were showing severe symptoms and in

Spurway, C. H. Soil testing—A practical system of soil diagnosis. Mich. Agr. Exp. Stat. Tech. Bull. 132 (Revised). 1935.

⁷ Acknowledgment is made to Dr. L. C. Wheeting for assistance in conducting the nutrient solution tests.

15 benches mild symptoms of yellows. No one condition or combination of conditions seemed to be a major factor in symptom development. Successive analyses of the soil throughout the growing season would be advisable if the relation of soil condition to symptom development is to be better understood.

CONTROL

Mosaic appears to be of little economic importance on carnations and since it is spread rapidly by mechanical contact in cultural practices there appears to be no practical method for its control. In the development of new varieties, care should be used to not handle the new seedling clones after handling commercial varieties.

Streak is transmitted readily by aphids, therefore the control of aphids is of the utmost importance in keeping this disease out of the plants. Because yellows is caused by transmission of the streak virus by aphids to plants already affected with mosaic, the control of aphids is very important if losses from yellows are to be avoided.

The mosaic and streak viruses are not transmitted in seed. Thus, new varieties developed from seed are free of mosaic, streak, and yellows. New varieties developed as sports from existing varieties are usually affected with the mosaic virus and often have yellows, depending upon the virus content of the mother plant. New varieties grown in the vicinity of commercial varieties, become affected with mosaic very rapidly when no care is used to prevent mechanical transmission of the virus in cultural practices. The rapidity of infection of new varieties with the streak virus depends upon the prevalence of this virus in adjacent stock and the transfer of aphids from diseased stock to plants of the new varieties.

Rogueing and selection of stock has not given complete control of yellows because the appearance of symptoms on diseased plants is not sufficiently distinct at any given time to allow for complete eradication. This was shown by an attempt to rogue yellows-affected stock for 2 growers in May, 1941. At benching time, stock showing no definite symptoms of yellows was selected from Joan Marie (2,065 plants) in one greenhouse and from King Cardinal (1,951 plants) in the other greenhouse. Observations on the plants in the resultant benches during the next season showed that a large number of infected plants escaped detection when the stock was rogued. Symptoms are usually more distinct as the cuttings come out of the sand or for a period of a month or 2 after removal from the sand. Careful selection of symptomless young plants from the time of removal from the sand until benching should be of great value in reducing losses from yellows. Symptoms on affected plants are usually so mild during the winter months that the selection of cuttings from only healthy plants appears impractical.

Growing carnations under optimal soil, fertility, and moisture conditions reduces losses from yellows. Unfavorable conditions that check the plant growth may increase symptoms and crop damage when the disease is present in the stock.

SUMMARY

Two viruses, mosaic and streak, are associated in the carnation disease known as yellows.

Mosaic shows as mottling of the leaves with light and dark green areas and slight color breaking in flowers of colored varieties with little damaging effect on the plant although generally present in all commercial varieties. Streak is characterized by yellowish to reddish spots and streaks on the foliage that may lead to death of portions of the plant. In commercial culture, streak is not observed alone but is in combination with mosaic to produce the disease known as yellows. The mottling of the foliage and breaking of color of the flowers caused by mosaic and the spotting of the foliage and death of portions of the plant caused by streak are intensified in the combination of the two viruses in the plant. Yellows has been very injurious to many varieties of carnations.

The mosaic virus is readily transmitted by mechanical inoculation but not by aphids or thrips, has a minimum period of incubation of 22 days, and is inactivated near 60° C. and after 42 days in vitro. The streak virus is transmitted by aphids but not by mechanical inoculation or by thrips, and it has a minimum period of incubation of 25 days.

Varieties of carnations differ in severity of damage caused by the yellows disease. The elimination of some varieties from commercial production, such as Betty Lou, Camelia, Chief Kokomo, Gardenia, Giant Laddie, Melrose, Patrician, Puritan, and Purity has been due largely to reduced production and lowered quality caused by yellows.

The 2 viruses were not transmitted in seed and considerable difference in susceptibility of seedlings was observed. Two virus-free and somewhat resistant seedling varieties are being tested in commercial greenhouses.

The development of symptoms of yellows is greatly influenced by environmental conditions. Unfavorable conditions that check plant growth, excess watering, low fertility, and excess salt in the soil, may enhance the development of symptoms.

The control of aphids is the most important practice to reduce losses from yellows. Careful selection of yellows-free planting stock is recommended, from the time the cuttings are removed from the sand until benching. The selection of yellows-free plants from which cuttings are removed does not appear to be practical since the symptoms of yellows are usually very mild during the winter months.

Washington Experiment Station, Pullman, Washington.

MYROBALAN MOTTLE AND ASTEROID SPOT

E. M. HILDEBRAND

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For the past five years two abnormalities, "mottle" and "asteroid spot," have been observed in myrobalan plum (*Prunus cerasifera*) seedlings used almost exclusively as root-stocks for propagating plums in fruit nurseries. Since World War II started in 1939, such seed comes almost exclusively from one nursery in California. Samples taken from shipments of seedlings from numerous nurseries as received in New York State have regularly disclosed the presence of these abnormalities during the growing season.

MOTTLE

Myrobalan mottle (Fig. 1) is characterized by an abnormal type of foliage development over the entire plant which is stunted. The leaves are small, irregular, undulating in surface, and contain dark green areas visible by reflected light.

Attempts to transmit mottle by budding or grafting from myrobalan plum to myrobalan plum, peach, cherry, and prune during 1941, 1942, and 1943 have been unsuccessful. On the other hand, the myrobalan mottle symptoms are regularly perpetuated on these plants in the growth from the affected buds. This tendency of bud perpetuation, but not of visible transmission, suggests a genetic phenomenon.

The origin of myrobalan mottle has been traced to the seed. Seed was obtained from the California nursery in 1942 and 1943 and grown in the greenhouse during the winter. Replicated plantings were made after variable periods of storage in moist peat at 5° C.¹ Each replicate consisted of 4 units of 40 or more seeds. Percentages of germination ranged from 90 to 100. It was discovered that at least one seedling had mottle symptoms in all replicated plantings of 120 or over of 1942 seed and of 50 or over of 1943 seed. Thus the percentage of seedlings affected with mottle ranged between 0.8 and 2.0 per cent in these two samples. These germination experiments leave no doubt that myrobalan mottle is seed-borne. When considered along with the fact that it is also bud perpetuated but not bud transmitted the conclusion is that myrobalan mottle is apparently a genetic abnormality originating at the seed source.

A variable percentage of myrobalan plum seedlings have been observed to contain the myrobalan mottle symptom inside and outside the State. In one case approximately 15 per cent of the seedlings showed this symptom in August although the percentage more commonly observed was between 0.5 and 5.0 per cent. No planting has been observed totally free from this abnormality.

 $^{^{1}}$ Usually a 3-month cold treatment was adequate for breaking dormancy of P. cerasifera.

ASTEROID SPOT

Asteroid spot or chlorotic spot describe a second abnormality on myrobalan plum, the first term describing the lesions on the foliage and the second those on the stems. At any time during the growing season few to many persistent star-shaped chlorotic spots (2 mm. \pm in diameter) may appear on the leaves (Fig. 2, A), and cause distortion (Fig. 2, B). The chlorotic spots

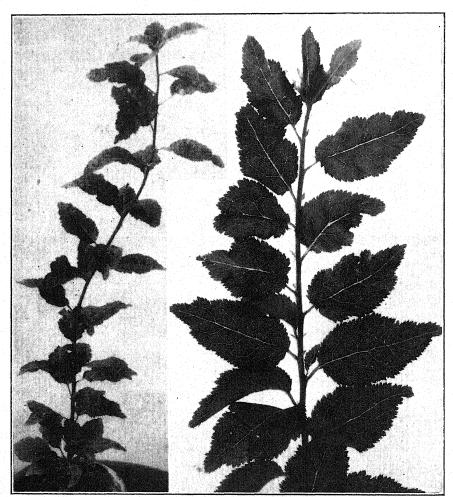


Fig. 1. Myrobalan mottle: left, young seedling plant; right, close-up view of foliage by transmitted light.

(5 mm. ±) on the stems are usually oval and may become necrotic although this is unusual. Every rootstock collection brought together thus far has had some asteroid spot. For example, in the 22 collections made in 1942 its presence ranged from occasional to 90 per cent. When symptoms were severe, root stock growth was stunted. In several experiments the percentages of plum buds surviving and growing the following season on severely

affected seedlings were considerably below, frequently less than 50 per cent, that in normal seedlings. The stem growth from Italian buds placed on affected plants was frequently stunted and symptoms on the foliage and stems were similar to those on the rootstock. On affected roots the annual growth from Italian buds was less than 2 feet in some cases, compared to 5 or 6 feet of growth on apparently normal roots.

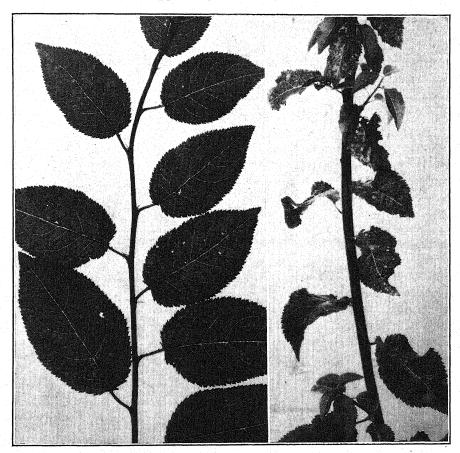


Fig. 2. Asteroid or chlorotic spot. On left, close-up of foliage in beginning stages showing few to many spots. On right, the advanced stage of spotting and distortion of the foliage accompanied by chlorotic spotting of the stem. Photographed by transmitted light.

Growing plants from seed in the greenhouse has practically excluded the chlorotic spot symptoms. However, since greenhouse temperatures run higher than those outdoors in spring and summer the possibility of temperature masking symptoms has not been excluded (2). When plants which showed symptoms outdoors in 1941, 1942, and 1943 were forced in the greenhouse the following winter no symptoms developed except on two plants which showed a few spots on the first growth made in early March. On neither the growth resulting from grafting with scions inserted during dor-

mancy nor from buds inserted during the growing season were symptoms later expressed at greenhouse temperatures. Grafting greenhouse seedlings with buds from severely affected plants in the field during the growing season has always given negative results. Symptomless seedlings grown from seed in the greenhouse when transferred to the field sometimes developed symptoms. At present the true cause of asteroid or chlorotic spot remains unknown.

The possibility that a certain insect may be the causative agent through injection of a toxin in its feeding operations has been probed. Thus far convincing evidence has not been obtained for or against this possibility. All attempts to transmit this abnormality to peach and other Prunus species have been negative.

DISCUSSION

No relationship seems to exist between myrobalan mottle and prune mottle (3) with which it might be confused. No relationship seems to exist between myrobalan asteroid or chlorotic spot and the necrotic asteroid spot virosis of peach reported from California (1).

Two abnormalities, mottle and asteroid or chlorotic spot, are described on myrobalan plum (Prunus cerasifera) root-stocks.

Myrobalan mottle, which commonly occurs in nursery plantings but affects only a small percentage of the seedlings, is a seedborne, bud-perpetuated abnormality presumably of genetic origin.

Asteroid or chlorotic spot which is of unknown etiology has been frequently encountered in nursery plantings where as high as 90 per cent of the seedlings may show symptoms. Severe symptoms result in stunting of both rootstock and scion growth.

CORNELL UNIVERSITY,

ITHACA, NEW YORK.

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THE PREVALENCE OF SEPTORIA ON CEREAL SEED IN CANADA¹

J. E. MACHACEK²

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In the autumn of 1937, the writer isolated from surface-sterilized Manitoba-grown wheat seed several cultures of a pale buff-salmon fungus. Pure cultures of this fungus produced, after an interval of several weeks, a few pycnidia on or near the surface of the culture. Spores typical of Septoria nodorum Berk. were formed in these pycnidia. Some of the wheat glumes present in the seed sample also carried pycnidia of S. nodorum.

In 1939, and in the following four years, a large number (6,183) of wheat, oats, and barley seed samples, collected from all the provinces of Canada, were subjected to a mycological examination. A part of the results from this investigation has already been published.³ From this seed, Sep-

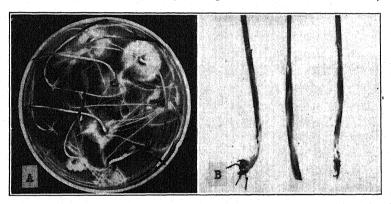


Fig. 1. Seed-borne Septoria nodorum. A. Fungus (light-colored colonies) emerging from surface-sterilized wheat seed. B. Lesions on coleoptiles of seedlings from infected wheat seed. Photo by A. M. Brown.

toria frequently was isolated. It was present most often in wheat seed and least often in oats seed. The relative prevalence of Septoria in these crops, as indicated by a plating-out test of surface-sterilized seed on potato-sucrose agar, in each of the eight seed-inspection districts of Canada is given in table 1, and the appearance of the fungus, as it emerges from surface-sterilized wheat seed, is shown in figure 1, A.

When wheat seed infected with Septoria was planted in nonsterile soil or sand in the greenhouse, many of the resulting seedlings showed elongate brown flecks or streaks on their coleoptiles (Fig. 1, B). With a few heavily infected lots of seed, some of the seedlings were distorted and, in addition to

¹ Contribution No. 788 from the Division of Botany and Plant Pathology, Science Service, Dept. of Agriculture, Canada.

Assistant Plant Pathologist.
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the lesions on the coleoptiles, bore a few small lesions on the leaves. Septoria nodorum was isolated from lesions on the coleoptiles and from lesions on the leaves. Lesions on the coleoptiles also developed in the field. When infected seed of oats and barley was planted in soil or sand in the greenhouse, the seedlings were free from lesions and were not distorted.

Coleoptile and leaf lesions, as well as stem distortion, were prevented when infected wheat seed was treated with New Improved Ceresan at the rate of one-half ounce per bushel, or with other organic mercury dusts at the recommended rates.

TABLE 1.—The relative prevalence of Septoria on the seed of wheat, oats, and barley in each of 8 seed-inspection districts of Canada during 1939-1943, and the percentage of infected seeds in the most severely infected seed samples in each district

		Mean percentage of Highest percentage of infected									
Inspection district	Crop	1939	1940	1941	1942	1943	1939	1940	1941	1942	1943
Maritime Provinces (Dist. 1)	Wheat Oats Barley	1.35 0.20 0.03	0.00 0.00 0.25	17.84 0.04 1.00	19.63 0.03 0.12	12.29 0.05 0.55	7 1 1	0 0 1	77 1 13	68 1 1	49 1 6
Quebec (Dist. 2)	Wheat Oats Barley	$0.57 \\ 0.00 \\ 0.14$	$0.00 \\ 0.04 \\ 0.13$	1.20 0.00 0.00	1.28 0.04 0.12	5.40 0.11 0.06	2 0 2	0 1 1	16 0 0	16 1 1	47 2 1
Eastern Ontario (Dist. 3)	Wheat Oats Barley	$0.00 \\ 0.12 \\ 0.00$	0.00 0.00 0.00	0.63 0.00 0.04	1.16 0.00 0.00	0.94 0.11 0.48	0 1 0	0 0 0	16 0 1	8 0 0	24 1 14
Western Ontario (Dist. 4)	Wheat Oats Barley	0.05	0.06 0.00 0.00	0.80 0.00 0.11	0.60 0.00 0.00	0.68 0.00 0.16	1	1 0 0	7 0 2	3 0 0	5 0 2
Manitoba (Dist. 5)	Wheat Oats Barley	$0.02 \\ 0.10 \\ 0.01$	0.07 0.01 0.06	0.01 0.00 0.00	0.74 0.12 0.50	1.09 0.49 1.09	2 1 1	3 1 3	2 1 0	12 2 10	8 4 21
Saskatchewan (Dist. 6)	Wheat Oats Barley	$0.03 \\ 0.03 \\ 0.04$		0.06 0.06 0.13	1.16 0.04 1.88	1.27 0.43 5.02	4 1 1		1 1 2	5 1 12	12 4 23
Alberta (Dist. 7)	Wheat Oats Barley	0.10 0.00 0.16		0.50 0.00 0.36	1.40 0.20 2.34	0.94 0.68 3.96	6 0 5		3 0 5	7 5 23	7 10 23
British Columbia (Dist. 8)	Wheat Oats Barley	0.06 0.12 0.00		0.00 0.00 0.00	0.66 0.00 0.09	0.63 0.11 0.45	1 1 0		0 0 0	6 0 1	3 2 2

In seed from the Maritime Provinces and from Quebec, more disease was present in wheat than in barley (Table 1). The opposite situation was found in the seed from Saskatchewan and Alberta, particularly in 1942 and 1943. This finding suggested the presence of two specialized strains or species of *Septoria* in the seed, one of which was more abundant in Eastern Canada and attacked primarily wheat while the other was more abundant on barley in Western Canada than on wheat.

A comparison of a large number of cultures derived from seed produced in both regions showed that the cultures from Eastern Canada generally produced pycnidia more abundantly than did those from Western Canada and that the surface mycelium of the former cultures was generally less cottony than that of the latter. During the early stage of growth, however, it was impossible to distinguish with certainty the two types of cultures.

A large number of cultures, derived from seed produced in both areas, were submitted to Dr. T. Johnson of this laboratory, for a critical examination. He found that the cultures from Western Canada, as a rule, produced pycnospores distinctly longer and thicker than those formed by the cultures from Eastern Canada. The size and appearance of the spores from the Western Canada cultures excluded the possibility that the fungus was either Septoria tritici Desm. or S. passerini Sacc., and tests with suitable hosts excluded S. avenae Frank. The majority of the cultures from Eastern Canada were identified as S. nodorum.

It is difficult to understand why Septoria nodorum has not been recognized earlier as a common seed-borne, disease-producing organism. From a study of cereal seed during the past five years, the fungus appears to be abundantly present in Eastern Canadian wheat seed—one seed sample from the 1942 crop, when sown in soil, gave rise to seedlings of which 88 per cent were lesioned. Rosen⁴ implied that S. nodorum was seed-borne, but did not actually prove it. The tardiness with which most of the cultures from seed sporulate probably has been one of the reasons why the occurrence of the fungus on seed has attracted so little attention.

DOMINION LABORATORY OF PLANT PATHOLOGY, WINNIPEG, MANITOBA, CANADA.

4 Rosen, H. R. Septoria glume blotch of wheat. Ark. Agr. Exp. Sta. Tech. Bull. 175. 1921.

SALTS AS ANTIDOTES TO COPPER IN ITS TOXICITY TO THE CONIDIA OF SCLEROTINIA FRUCTICOLA¹

PAUL B. MARSH²

(Accepted for publication August 10, 1944)

The conidia of Sclerotinia fructicola are very sensitive to the fungicidal action of copper. Lin (4) has reported, however, that the toxicity of copper sulphate to these spores may be strikingly decreased by any one of several inorganic salts. The data presented here confirm the existence of Lin's "copper antidoting" phenomenon and provide certain new information concerning it.

High germination of the conidia of *S. fructicola* brought about by addition of an antidoting salt to a copper-sulphate-containing medium has been found to be associated with a decrease in the amount of copper absorbed by the spore. The protective phenomenon may be observed also in the respiratory behavior of the spores. Data on these and related points are discussed from the standpoint of the physiology of spore germination and the mechanism of the toxic action of copper.

MATERIALS AND METHODS

Three different measurements have been made on the conidia of *Sclerotinia fructicola*—percentage germination, respiratory rate, and copper absorption. Spore-germination experiments were in Pyrex Petri dishes containing glass-distilled water, copper sulphate, glucose, spores, and in some cases, an antidoting salt. Spores for these experiments were obtained by adding a little water to 10–14-day-old Petri-dish cultures on potato-dextrose agar, dislodging the spores with a camel's hair brush, filtering through Kleenex to remove hyphal fragments, and washing the spores by three successive centrifugations in 50-ml. portions of glass-distilled water. Spore germination counts were made after 20 hours at 26° C.

In the spore germination experiments (Table 1), spore concentrations were adjusted to approximately 25,000 spores per ml. In the copper absorption and respiration experiments, the concentrations ranged from 300,000 to 1,000,000 per ml. In early experiments, a Levy counting chamber was used to determine spore concentrations. A more rapid method adopted in later experiments utilized the measurement of light absorption by the suspensions in a photoelectric colorimeter.

In determining the rate of oxygen consumption by the spores, the Fenn (1) micro-respirometer was used. This instrument consists essentially of a small glass experimental vessel which is closed to the atmosphere when in operation but connected with a graduated capillary containing an index drop which is free to move in response to the consumption of oxygen by the

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² The writer wishes to acknowledge his indebtedness to Prof. V. L. Frampton for helpful comments during the course of this study.

respiring tissue. The carbon dioxide of respiration is absorbed by KOH in an inset in the experimental vessel. The micro-respirometers used had a volume of 11 ml. in the experimental vessels and capillary volumes close to 3.5 mm.³ per linear centimeter. They were rocked in a water bath held at a constant temperature of 26° C.

Except where otherwise indicated, copper was determined colorimetrically by the use of sodium diethyl dithiocarbamate. The reagent has been studied by Greenleaf (3) and others. In determining copper in the supernatant fluid after germination, 5 ml. of the supernatant fluid was pipetted into the 10-ml. absorption cell of a Cenco-Sheard-Sanford photelometer. To this was added 1 ml. of a 0.01 per cent carbamate reagent, and glass-distilled water to a total volume of 10 ml. The concentration of copper was determined from a previously prepared calibration curve. Determination of copper by this method was very satisfactory over a range of concentra-

TABLE 1.—Effect of single salts in antidoting the inhibitory effect of copper sulphate on germination of the conidia of Sclerotinia fructicola

Salt conc.	Percentage germination in 4×10^{-7} M CuSO ₄ plus 0.01 per cent glucose and:						
	MgSO ₄	CaCl ₂	KCl				
0 10 ⁻⁵ M 10 ⁻⁴ M	1.2 54.0 67.0	0.8 31.0 62.0	2.9 3.9 2.6				
10 ⁻³ M 10 ⁻² M	78.0	83.0	3.9 59.0				

tions from 10^{-5} M to 10^{-4} M, *i.e.*, from about 0.6 gamma per ml. to 6 gamma per ml. Copper determinations with potassium ethyl xanthate were according to the same procedure used with the dithiocarbamate.

In determinations of the copper content of the conidia themselves, the spores were first ignited in porcelain crucibles at a temperature below red heat in a muffle furnace. After the crucibles had cooled, a small amount of concentrated nitric acid was added; this was evaporated off on a hot plate and replaced by about 2 ml. of concentrated ammonia. After 4 or 5 hours, water and carbamate reagent were added and the determination completed.

Water used in the germination medium and in respiration and copper absorption determinations was prepared by redistillation of ordinary distilled water in a Pyrex glass still. The glucose used was prepared by recrystallization of Baker's reagent grade glucose. All other chemicals were of reagent grade. All glassware was cleaned in dichromate-sulphuric acid cleaning solution and rinsed thoroughly in tap water, distilled water, and glass-distilled water before use.

EXPERIMENTAL RESULTS

Table 1 presents the results of 3 spore-germination experiments and demonstrates the protective action of salts in the presence of copper sulphate.

TABLE 2.—Magnesium sulphate protects conidia of Sclerotinia fructicola against poisoning of respiration by copper sulphate; antagonizes effect of copper sulphate on germination

		Cubic mm. O2 consumption per million spores					
	Hour	Control	In 2×10-4 M CuSO ₄	$\begin{array}{c} \text{In } 2\times10^{-4}\text{M CuSO}_4\\ \text{plus } 2\times10^{-2}\text{M}\\ \text{MgSO}_4 \end{array}$			
st nd rd th th th		7.87 9.06 9.26 9.65 9.70 9.16 9.11	3.18 1.68 2.05 1.49 1.14 0.86 0.93	5.01 4.37 4.15 4.82 4.95 5.00 5.00			
		Percer	atage germination after	8 hr.			
		95	0	40			

Each germination plate contained 4×10^{-7} M copper sulphate and 0.01 per cent glucose; the salt concentrations ranged from zero to as high as 10^{-2} M for the KCl. It will be noted that mol for mol KCl was less effective as a protective agent than either MgSO₄ or CaCl₂.

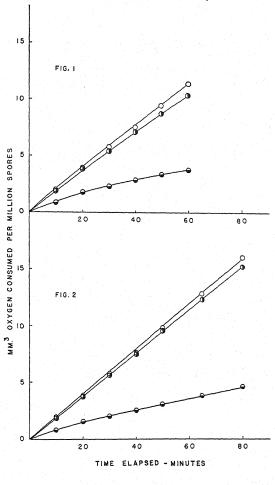
Figures 1 and 2 and table 2 present the protective phenomenon as it may be observed in the respiratory rates of the spores. Equal amounts of a conidial suspension in 1 per cent glucose were placed in each of three Fenn (1) micro-respirometer vessels. Copper sulphate was added to one vessel and copper sulphate plus magnesium sulphate to a second vessel, while the third vessel was left as a control, containing neither copper sulphate nor added salt. Magnesium sulphate protects the conidia against respiratory poisoning by copper sulphate. Table 3 shows that calcium chloride exerts a similar protective action.

When supplied in suitable concentration, calcium chloride or magnesium sulphate will partially, or in some cases almost completely, prevent the ab-

TABLE 3.—Calcium chloride protects conidia of Sclerotinia fructicola against poisoning of respiration by copper sulphate; antidotes the effect of copper sulphate on germination

	Cubic mm. O ₂ consumption per million spores					
Hour	Control	In 2×10^{-4} M CuSO ₄	$\begin{array}{c} \text{In } 2\times10^{-4}\text{M CuSO}_4\\ \text{plus } 2\times10^{-2}\text{M}\\ \text{CaCl}_2 \end{array}$			
1st	9.66 8.19 8.00 9.95 9.49 9.39	1.70 1.72 1.63 1.17 0.85 1.03	6.17 5.75 6.62 8.31 8.06 7.31			
	Percen	tage germination after	18 hr.			
	93	0.02	87			

sorption of copper from a copper sulphate solution by conidia of the brown-rot fungus. This fact is clearly apparent from the data in figure 3. The experiments whose results are shown in figure 3 were carried out by adding to each of a pair of Pyrex Petri dishes 5 ml. of a spore suspension in 2 per cent glucose, then 1 ml. of 0.1 M CaCl₂ (or MgSO₄) to one of the plates, and



O CONTROL • .0001 M. COPPER SULPHATE

O .0001 M. COPPER SULPHATE PLUS .01 M

MAGNESIUM SULPHATE

Figs. 1 and 2. Magnesium sulphate protects conidia of Sclerotinia fructicola against respiratory poisoning by copper sulphate.

finally 1 ml. of 10⁻³ M CuSO₄ and water to a total volume of 10 ml. to each plate. After 20 hours, the spores were centrifuged out of suspension and copper determinations made with the dithiocarbamate reagent. In experiments C-1 to C-10 inclusive, copper determinations were made on the supernatant fluid remaining over the spores after centrifuging the suspensions.

In experiments C-11 to C-14 and M-11 and M-12, copper analyses were made on the spores themselves. Spore germination in the copper absorption experiments was in all cases very much higher in the presence of the antidoting salt than in its absence. The germination figures for experiments C-1 to C-10 were 91, 95, 90, 95, 90, 92, 80, 64, 90, and 88 per cent for the "plus salt" plates and 2, 1, 1, 2, 0, 1, 0, 0.5, 1, and 0 per cent for the corresponding "minus salt" plates; similarly in the M series, germination percentages in the "plus salt" plates were 92, 96, 93, 84, 86, 91, 90, 92, 86, and 89

CALCIUM CHLORIDE SERIES

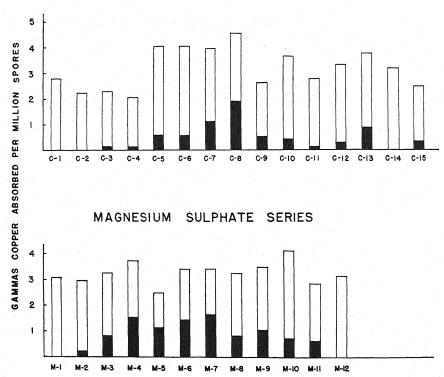


Fig. 3. Calcium chloride and magnesium sulphate depress the absorption of copper by conidia of S. fructicola from a copper sulphate solution. Total bar shows copper absorbed in absence of salt, blackened portion the same in the presence of salt.

as compared with 0, 1, 0, 0, 0, 0, 1, 1, 2, and 1 in the corresponding "minus salt" plates.

Salt depression of copper absorption may be observed after a very short absorption interval. In two 10-minute experiments, carbamate analysis of the supernatant fluid resulted in values of 0.36 and 0.45 gammas of copper absorbed per million spores in the presence of 10^{-2} M CaCl₂ and 10^{-4} M CuSO₄ as compared with 2.50 and 2.30 gammas absorbed in the presence of the copper sulphate alone. Similar experiments with MgSO₄ yielded values of 0.49 and 0.51 gammas absorbed in the presence of the 10^{-2} M MgSO₄ and

10⁻⁴ M CuSO₄ as compared with 1.59, 2.39, and 3.12 gammas absorbed in the absence of the protective salt. The 10-minute experiments differed from those of figure 3 in that no glucose or other carbon or energy source was supplied. The phenomenon of salt depression of copper absorption is thus rapid and not dependent on an external energy source for the spores. In the 10-minute experiments, the Petri dishes were rocked gently so that diffusion of copper in the solution would not limit the rate of copper absorption.

Experiments early in the course of the work had shown that neither CaCl₂ nor MgSO₄ interfered in the carbamate determination of copper; it was easily demonstrated further that when a known amount of copper sulphate was added to a supernatant fluid recovered from a spore suspension which had germinated in the presence of copper sulphate and either CaCl₂ or MgSO₄, this known increment of copper could be closely checked by carbamate copper analysis. As a final check, however, experiments were

TABLE 4.—Effect of potassium chloride on the absorption of copper from a copper sulphate solution (10- 4 M) by conidia of Sclerotinia fructicola

		Copper absorption and percentage germination with KCl					
TD NT	Molarity of	Pres	sent	Absent			
Exp. No.	KCĬ	Gammas Cu per million spores	Per cent germination	Gammas Cu per million spores	Per cent germination		
K-1 K-2 K-3 K-4	0.01 0.1 0.5 1.0	2.22 2.82 0.51 0.0	0 0 5 78	2.62 4.06 3.10 4.36	0 0 0 0		

carried out in which an entirely different reagent was used for determining copper, namely potassium ethyl xanthate. In three 10-minute absorption experiments, using 10⁻² M CaCl₂ as antidote to 10⁻⁴ M CuSO₄, the copper absorption was 8.10, 4.49, and 4.14 gammas per million spores in the absence of the salt and essentially zero, or below the limit of determination, in its presence. In similar experiments with 10⁻² M MgSO₄, the absorption values were 6.89 and 8.86 gammas in the absence of salt and zero in its presence.

Table 1 shows that the minimum effective ratio of KCl to CuSO₄ which is required for antidoting action to be observed in spore germination differences is very much higher than the same ratio for CaCl₂ or for MgSO₄. Similarly, the minimum effective ratio of KCl to CuSO₄ required to influence copper absorption is very high. Results with a range of concentrations of KCl are in table 4. Germination in the higher concentrations of KCl is somewhat slower than ordinary, but the germ tubes are normal in appearance.

DISCUSSION

The absorptive capacity of the conidia of *Sclerotinia fructicola* for copper in terms of copper per unit volume of spore is high. It is thus possible for conidia of this fungus in contact with a dilute copper sulphate solution to

absorb a large proportion of the copper from the surrounding liquid medium. This fact, in conjunction with the circumstance that the actual quantity of living material per unit volume in spore suspensions of the density ordinarily used in spore germination work is extremely low, offers at least a partial explanation of the extraordinarily low concentrations of copper which are effective in preventing germination of the conidia of *S. fructicola*. In experiment C-1, for example, the total number of gammas of copper present in the "minus salt" dish was 62.5. At the end of the experiment 19.2 gammas of copper had moved into the spores, whose total volume was only about 0.22 per cent of the total volume of the suspension. The concentration of copper per unit volume of spores at the end of the experiment was thus 1,930 times as great as in the external medium. Similar calculations for experiments C-1 to C-10 and M-1 to M-10 revealed that in some cases the concentration of copper in the spores was as much as 4,000 times as high as in the external medium.

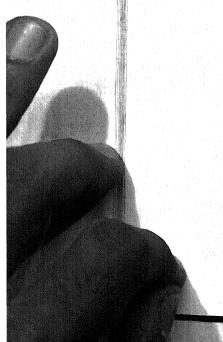
When a salt presented singly to an organism inhibits its normal growth or activity but this inhibitory effect is diminished by addition of a second salt, the phenomenon observed is usually called ion antagonism. According to this definition, the copper-antidoting phenomenon here described is a case of ion antagonism. While much has been written concerning a relation between ion antagonism and ion absorption, actual experimental data on the point are very meager. The data here presented are thus of theoretical interest from the standpoint of the understanding of ion antagonism reactions in general.

While salts such as MgSO₄, CaCl₂, and KCl antidote the toxic action of copper toward the conidia of S. fructicola, it appears from the experiments here reported that these conidia do not require the addition of such salts or indeed of any other materials beside pure glucose and pure water to enable them to germinate in the absence of toxic materials. In this respect the author's results are at variance with those of McCallan and Wilcoxon (5) and those of Goldsworthy and Green (2). The cause of this difference in results has not been ascertained and might be difficult to prove. The possibility cannot be overlooked, however, that traces of toxic metals were present in the ordinary distilled water used by these investigators and that the function of orange juice or other natural decoctions necessary to secure high germination in the check plates was related to their content of heavy-metal-inactivating salts and proteins.

SUMMARY

Experiments are described which deal with the toxic action of copper sulphate on the conidia of *Sclerotinia fructicola*. It is shown that calcium chloride, magnesium sulphate, or potassium chloride may counteract or antidote the inhibitory effect of copper sulphate on the germination of these spores; this confirms the observations of Lin (4).

The copper-antidoting action of salts may be observed in the respiratory rates of the spores.



High germination of the spores brought about by the protective action of salts is associated with decreased copper absorption.

The spores of this fungus have a very high affinity for copper, being able to concentrate it within themselves from a very dilute solution.

PLANT INDUSTRY STATION. BELTSVILLE, MARYLAND.

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SPECIALIZATION OF PATHOGENICITY IN ERYSIPHE GRAMINIS ON POA AND ITS RELATION TO BLUEGRASS IMPROVEMENT¹

JOHN R. HARDISON2

(Accepted for publication August 18, 1944)

In a previous paper (1) the results of inoculation experiments with Erysiphe graminis DC. were reported in which fungus cultures from grasses in the tribe Hordeae infected only grasses in this tribe. Furthermore, each culture infected species of two or more genera, thus demonstrating that races of E. graminis are not necessarily restricted to the species of any one genus as had been previously supposed for the most part. The present paper reports the results of inoculation experiments with five cultures of the fungus from species of Poa.

Marchal (4) described the specialized variety, Erysiphe graminis poae, as infecting different Poa species, notably P. annua, P. caesia, P. mutalensis, P. nemoralis, P. pratensis, P. serotinia, and P. trivialis. Conidia from P. annua and P. pratensis did not infect Hordeum vulgare. Marchal inferred that E. graminis poae did not infect Avena sativa, Bromus species, Hordeum vulgare, Secale cereale, or Triticum vulgare.

Salmon (6) reported that conidia from Poa pratensis produced infection on P. pratensis and sub-infection on Poa annua and P. nemoralis. There was no infection of Agropyron repens, Alopecurus pratensis, Avena sativa, Dactylis glomerata, Festuca elatior var. pratensis, F. arundinacea, F. heterophylla, Hordeum vulgare, Lolium perenne, L. temulentum, Phleum pratense, Secale cereale, or Triticum vulgare.

Reed (5) states that conidia from Poa pratensis infected P. compressa, P. nemoralis, P. pratensis, and P. trivialis but did not infect Avena sativa, Bromus mollis, Dactylis glomerata, Festuca elatior, Hordeum jubatum, H. vulgare, Lolium perenne, Phleum pratense, Secale cereale, or Triticum vulgare. Reed also suggested that there may be more than one specialized race within E. graminis poae.

Unpublished notes taken by the writer in grass nurseries at Ann Arbor, Michigan, and Pullman, Washington, record several instances of mildewsusceptible accessions of Poa species remaining uninfected while growing in rows adjacent to heavily mildewed bluegrass plants of the same as well as of

Part of this investigation was made at the University of Michigan in studies leading to the degree of Doctor of Philosophy in Botany, and part at the Kentucky Agricultural Experiment Station. It is published by permission of the Director of the Kentucky Agri-

² The writer gratefully acknowledges the suggestions and criticisms given by Dr. E. B. Mains; seed of the various grasses supplied by many collaborators; the cooperation of Dr. George W. Fischer in sending cultures of the fungus, and the assistance of Mr. J. R. Swallen in checking the identity of the majority of the grasses used.

TABLE 1.—The number of accessions of various grass species infected after inoculation with 5 cultures of Erysiphe graminis from 3 species of Poa

		Culture N	o. and sour	ce species				
Species testeda	P. pratensis	$P. \\ ampla$	P. palustris	P. pratensis	P. pratensis			
	1	14	20	21	22			
	Number of accessions infected ^b							
Aegilops crassa Boiss.	0/2	0/1	T					
A. cylindrica Host	0,2	0/1		0/1				
A. triuncialis L.	0/1				********			
Agropyron caninum (L.) Beauv		********		0/1				
A. cristatum (L.) Beauv.	0/4	0/4	0/1	0/4				
A. desertorum (Fisch.) Schult	0/1	0/1	0/1	0/1	••••••			
Rydb.	0/12	0/11	0/4	0/1	********			
A. intermedium (Host) Beauv	0/1		0/1	0/1	*********			
A. repens (L.) Beauv.	0/1		0/1	0/1	*********			
A. semicostatum (Steud.) Nees	0/1	0/1	0/1	0/1				
A. sibiricum (Willd.) Beauv	0/5	0/5	0/2	0/5	*******			
A. spicatum (Pursh) Scribn. and	0/1	0/1	0/1	0/1				
Smith	0/13	0/11	0/2	0/12				
A. striatum (Steud.) Nees ex Hook.	0./1	0/1		0/1	*********			
A. subsecundum (Link) Hitche	0/1	0/1	0.79	0/1	*******			
A. trachycaulum (Link) Malte	0/13	0/14	0/3	0/13				
Agrostis alba L.	0/4	0/4	0/4	0/4	0/5			
A. exarata Trin.	0/1	0/1	0/1	0/1	0/1			
A. hiemalis (Walt.) B.S.P. A. palustris Huds.	0/1	0/1	0/1	0/1 0/1	0/1			
A. scabra Willd.		0.41	*********	0.41	0/1			
A. spica-venti L. A. stolonifera L.		0/1 0/1	********	0/1				
Alopecurus aequalis Sobol		0/1		0/1	0/1			
A. pratensis L.		0/3	0/1	0/2	0/2			
Anthoxanthum odoratum L					0/1			
Arrhenatherum elatius (L.) Mert.	0/1	0/1	0/1	0/1	0/1			
Avena sativa L.	0/1	0/1	0/1					
	1			21.7				
Beckmannia erucaeformis (L.) Host B. syzigachne (Steud.) Fernald	0/1 0/1	0/1	0/1 0/1	0/1 1/1	0/1 0/1			
Bromus arvensis L.	0/2	0/2		0/2				
B. brizaeformis Fisch. and Mey	0/1	0/1		0/1				
B. carinatus Hook. and Arn.		0/2	0/2	0/2				
B. catharticus Vahl	0/3	0/3		0/3				
B. commutatus Schrad.	0/1	0/1	0.40	0/1				
B. inermis Leyss.	0/6	0/6	0/2	0/6				
B. japonicus Thunb.	0/2	0/2		0/2				
B. macrostachys L.	0/2	0/2	********	0/2	********			
B. macrostachys var. lanuginosus	0.77	0./1		0./1				
Boiss.	0/1	0/1	0.79	0/1	**********			
B. marginatus Nees	0/7	0/8	0/2	0/8	********			
B. mollis L.	0/2	0/2	*********	0/2	***************************************			
B. polyanthus Scribn.	0/1	$0/1 \\ 0/2$	*********	0/1 0/2	************			
B. purgans L.	0/2		PHONO	0/1	********			
B. rigidus Roth	0/1	0/1	*********	0/1	***************************************			
B. rubens L. (Schrad)	0/1	0/1	Manico	0/1	**********			
B. secalinus var. velutinus (Schrad.) Koch	0/1	0/1	. mana	0/1				
B. squarrosus L.	0/1	0/1	*********	0/1				
B. tectorum L.	0/1	0/1	********	0/1	********			

TABLE 1—(Continued)

		Culture N	o. and sour	ce species				
Species testada	P. pratensis	P. $ampla$	P. palustris	P. pratensis	P. pratensis			
Species tested	1	14	20	21	22			
	Number of accessions infected ^b							
eschampsia caespitosa (L.) Beauv. danthonioides (Trin.) Munro lymus canadensis L. condensatus Presl dahuricus Turez. glaucus Buckl. junceus Fisch. sibiricus L. triticoides Buckl. villosus Muhl. virginicus Var. glabriflorus (Vasey) Bush virginicus var. intermedius (Vasey) Bush estuca elatior L. elatior var. arundinacca (Schreb.) Wimm. gigantea (L.) Vill. idahoensis Elmer obtusa Spreng. occidentalis Hook. octoflora Walt. virubra L. rubra L. rubra Var. commutata Gaud. seabrella Torr. thurberi Vasey viridula Vasey	0/1	0/1		0/1				
	0/1	0/1		0/1				
Dactylis glomerata L.	0/8	0/8	0/8	0/8	0/8			
	1/1	0/1	0/2	0/2	0/2			
D. danthonioides (Trin.) Munro		1/1		1/1	0/1			
Elymus canadensis L.	0/5	0/4	0/2	0/5				
E. condensatus Presl	0/4	0/4	0/1	0/4				
E. dahuricus Turcz.	0/1	0/1	0/1	0/1				
	0/4	0/3		0/3	********			
	0/1	0/1		0/1				
	0/1	0/1 0/1	0/1	0/1	*******			
		0/1	0/1					
	0/3	0/4		0/4				
E. virginicus var. glabriflorus (Vasey) Bush	0/1	0/1						
/ W		0.71						
	0.43	0/1	***************************************	0.43				
F. elatior var. arundinacea (Schreb.)	0/1	0/1		0/1	0/1			
Wimm.	0/1	0/1		0/1	0/1			
F idahoensis Elmor	0/1	0/1	0/1	0/1	0/1			
		0/1	0/1	1/1	0/1			
		1/1		1/1				
F. octoflora Walt.		1/1		1/1	0/1			
F. ovina L.		0/1						
F. rubra L.	1/1	0/2		0/1	0/1			
F. rubra var. commutata Gaud	1/1	0/1		1/1	0/1			
		0/1						
		1/1		1/1	0/1			
	0.44	0/1						
	0/1	0/1		0/1	0/1			
		0/1						
	*******	0/1	**********	0/1				
		********		0/1				
4	0/1	0/1						
H. murinum L.	0/1			0/1	********			
H. nodosum L.		,		0/1				
H. vulgare L.	0/2	0/2	0/2	0/2				
Hystrix patula Moench			0/1					
Koeleria cristata (L.) Pers	3/5	3/7	0/6	2/4	0/5			
Lolium multiflorum Lam.	0/1	0/1	0/1	0/1				
L. perenne L.	0/1	0/1	0/1	0/1				
Milium effusum L.		0/1	********		0/1			
Phalaris arundinacea L.	1/1	0/1	0/1	0/1	0/1			
Phleum pratense L.	2/3	0/3	0/3	3/3	0/3			
Poa ampla Merr.	3/7	21/30	0/7	0/30	0/13			
P. arachnifera Torr.		0/1		1/1	0/1			
P. arctica R. Br.	1/1	1/1		1/1	0/1			
P. arida Vasey		1/1		0/1	0/1			
P. bulbosa L.	1/1	2/4	0/1	0/4	0/6			

TABLE 1—(Continued)

		Culture N	o. and sour	ce species				
Species testeda	P. pratensis	P. ampla	P. palustris	P. pratensis	P. pratensis			
	1	14	20	21	22			
	Number of accessions infected ^b							
P. canbyi (Scribn.) Piper	0/3 2/3	6/8 2/3	0/3 1/3	0/8 2/3	0/5 0/5			
P. curta Rydb. P. cusickii Vasey	1/1 1/1	$0/1 \\ 1/2$	0/1 1/1	1/1 2/2	0/1 1/1			
P. epilis Scribn	$\begin{array}{c c} 1/1 \\ 1/2 \end{array}$	1/2 3/3	0/1 0/2	$1/1 \\ 1/3 \\ 0/1$	0/2 0/2 0/1			
P. interior Rydb	0/2 1/3	$0/1 \\ 4/5 \\ 3/7$	0/2 0/1	0/1 0/5 0/6	0/1 0/6 0/8			
P. nervosa (Hook.) Vasey P. nevadensis Vasey	2/3	0/2 6/6	0/3	0/2 0/6	0/2 0/4			
P. palustris L. P. pratensis L.	$\frac{1/2}{11/12}$	$\frac{2}{6}$ $\frac{1}{16}$	4/4 2/11	$\frac{2}{7}$ $\frac{14}{15}$	2/4 42/88			
P. scabrella (Thurb.) Benth.	0/1 2/3 2/3	3/3 6/6 5/5	0/1 0/3 0/3	0/3 0/6 1/4	0/3 1/8 1/5			
P. sphondyloides Trin. P. sterilis Bieb. P. trivialis L.		0/1		1/1	1/1 1/2			
Polypogon monspeliensis (L.) Desf.		1/1	0/1 0/1	0/1 1/1	0/1 0/1			
Puccinellia distans (L.) Parl Secale cereale L	0/1	0/1	0/1	0/1				
Sitanion hystrix (Nutt.) J. G. Smith	0/1	0/1	0/1	0/1				
S. jubatum J. G. Smith	0/1	0/1 0/1	0/1 0/1	1/1	0/1			
T. spicatum (L.) Richt.	0/1	0/1 0/1	0/1	1/1 0/1	0/1			

^a Hitchcock (2) was followed wherever possible for the nomenclature of the grasses.
^b Numerator of fraction refers to number of accessions infected; denominator refers to total number of accessions tested. The term accession is explained under Materials and Methods.

MATERIALS AND METHODS

Selected groups of 398 accessions of the grass species listed in table 1 were used. The methods of handling fungus cultures and grasses have been described in detail in another paper (1). Grass seedlings were inoculated by atomizing their leaves with an aqueous suspension of fresh conidia and covering the inoculated plants with wet muslin for 24 to 48 hours. The classes of reaction used in recording notes were those of Mains and Dietz (3, p. 231).

The term "grass accession" in this paper refers to a numbered collection which represents a sample of the population of any one grass species. In most cases, these collections are genetically variable. Many of them were obtained from the Soil Conservation Service and represented seed produced from their collections grown in row nurseries. The specific identifications of these grass accessions were checked by the personnel of the Soil Conservation Service. Recognizing the possibilities of error both in previous identifi-

cations and in harvesting and preparation of nursery seed samples, a number of plants of all grass accessions used were grown to maturity. Representative samples of most accessions were sent to Mr. J. R. Swallen, Division of Plant Exploration and Introduction, U. S. Department of Agriculture, for verification. Likewise, careful personal attention was given to the gross morphology of both adult and seedling plants. The possibility of species mixtures was under constant surveillance, and particularly when unusual infection results were obtained. These precautions should give reasonable assurance of accuracy in regard to the specific identity of the grasses.

RESULTS

In table 1 are the general results of inoculating from 1 to many accessions of 116 species and 8 varieties of grasses with 5 cultures of Erysiphe graminis from 3 species of Poa. These results demonstrate that powdery mildew from Poa is not necessarily restricted to species of this genus. The large-scale negative results permit a clearer understanding of the host range of Poa mildews. The grass accessions listed as infected in table 1 include all cases where definite infection was recorded.

Distinction is made between resistant and susceptible types of reaction of grass accessions in table 2. The 38 accessions of various grass species listed in table 2 were chosen because their reactions are most significant in illustrating differences in the pathogenicity of the 5 mildew cultures and in the host range of Poa mildews. If only resistant types of reaction are found in grass accessions of certain grass species, these may be important clues to a more complete understanding of the host range, since other untested collections of the species may be very susceptible. This is especially true when a restricted number of collections of grass species are tested.

Culture 1. On Poa pratensis, from Ann Arbor, Michigan

Many species of *Poa* were infected by culture 1 and, in addition, *Deschampsia caespitosa*, *Festuca rubra*, *F. rubra* var. *commutata*, *Koeleria cristata*, *Phalaris arundinacea*, and *Phleum pratense*. The largest number of susceptible bluegrasses are among the related species of the *Pratenses* and *Epiles* groups of the genus *Poa*.³ Culture 1 was neither as vigorous nor as destructive as cultures 21 or 22 also from *P. pratensis*.

Culture 21. On Poa pratensis, from Mandan, North Dakota

Culture 21 was collected by Dr. Roderick Sprague and the writer at the Northern Great Plains Field Station. This vigorous culture infected more accessions and species of *Poa* as well as more species of other genera than did culture 1. Besides bluegrasses, infection was produced on species of *Beckmannia*, *Deschampsia*, *Festuca*, *Koeleria*, *Phleum*, *Puccinellia*, and *Trisetum*. Susceptible collections of bluegrasses were primarily among the related species of the *Pratenses* group of the genus *Poa*.

³ The taxonomic subsections of the genus Poa are taken from Hitchcock (2).

TABLE 2.—Reactions of 38 accessions of grasses to 5 cultures of Erysiphe graminis from 3 species of Poa

Grass accessionsa	P. pratensis	$P. \\ ampla$	P. palustris	P. pratensis	$\begin{array}{c} P. \\ pratensis \end{array}$	
	1	14	20	21	22	
		Typ	es of react	ionb	<u>'</u>	
Agrostis exarata 21	0	0	0	0	0	
Agrostis palustris 22	0	0	0	0	0	
Alopecurus pratensis 234		0		0	0	
Arrhenatherum elatius 23	0	0	0	0	0	
Avena sativa 369	0	0	0	0		
Beckmannia erucaeformis 11	0	0	0	0	0	
Beckmannia syzigachne 12	0	0	0	2	0	
Bromus carinatus 229		0	0	0		
Dactylis glomerata 31	0	0	0	0	0	
Deschampsia caespitosa 9	2-		0	0	0	
Deschampsia caespitosa 10	0	0	0	0	0	
Deschampsia danthonioides 318		2		2	0	
Festuca idahoensis 250		0	0	2	0	
Festuca octoflora 323		3 +		2+	0	
Festuca rubra var. commutata 85	2	0		4	0	
Holcus lanatus 187	0	0		0	0	
Koeleria cristata 32	0	0	0	0	0	
Koeleria cristata 33	3 -	2+	0	2+	0	
Koeleria cristata 34	2+	2 -	0	0	0	
Milium effusum 223		0			0	
Phalaris arundinacea 13		0	0	0	0	
Phleum pratense 15		0	0	2+	0	
Phleum pratense 16		0	0	2-	0	
Poa arachnifera 224		0		4	0	
Poa canbyi 43		4	0	0	0	
Poa compressa 49	1	2-	2+	2+	0	
Poa compressa 50	4	0	0	4	0	
Poa curta 51	4	0	0	4	0	
Poa nevadensis 59		3	0	0	0	
Poa palustris 57		Ō	3	0-1	0	
Poa palustris 65	1 -	1+	3+	0	0	
Poa pratensis 68		ō	0	2	0-1	
Poa pratensis 69	1	o o	0	0	0	
Poa pratensis 70	_	Ŏ.	0	4	0-1	
Polypogon monspeliensis 230		ő	0	0	0	
Puccinellia distans 251		4	0	4	0	
Trisetum flavescens 228		Ô		2	0	
Trisetum spicatum 329		ő		2	0	

² Accession numbers assigned to grass collections by the writer.

^b Classes of reaction expressed according to the system of Mains and Dietz (3):

0—Highly resistant. Little or no mycelium. Chlorotic or necrotic spots on some hosts.

-Very resistant. Slight to moderate mycelium. No sporulation. Chlorotic or

necrotic spots may develop.

2—Moderately resistant. Moderate to abundant mycelium. Slight sporulation.

Chlorotic or necrotic areas may develop.

3—Moderately susceptible. Moderate to abundant mycelium. Moderate sporulation.

4-Very susceptible. Abundant mycelium and sporulation.

-Indicates no test was made.

Culture 22. On Poa pratensis from Lexington, Kentucky

Culture 22 was collected from plants in a well-established sod where the development of mildew resulted in severe defoliation during the fall and early winter of 1942. In the spring of 1943 very little new growth appeared in the affected small area. Although the sod reached a fair development by midsummer, the loss of spring growth was substantial.

Culture 22 infected fewer bluegrass species than cultures 1 and 21. No infection was produced outside of the genus *Poa*. As with culture 21, infected species are largely in the *Pratenses* group of the genus *Poa*.

Culture 14. On Poa ampla from Pullman, Washington

Culture 14 was started from infected plants sent by Dr. George W. Fischer. It infected many collections of species of *Poa* and also *Deschampsia danthonioides.*, *Festuca occidentalis*, *F. octoflora*, *F. thurberi*, *Koeleria cristata*, and *Puccinellia distans*. Bluegrass species showing the greatest susceptibility to this culture are among the *Scabrellae* and *Nevadenses* groups of the genus *Poa*.

Culture 20. On Poa palustris from Pullman, Washington

Only collections of *Poa palustris* were moderately or very susceptible to culture 20. Resistant types of reaction were recorded on one accession each of *Poa compressa* and *P. cusickii* and two accessions of *P. pratensis*. The fact that no other bluegrasses were infected among the many species tested indicates that this culture may be rather closely restricted to *P. palustris*.

The pathogenically different cultures may be easily distinguished on one accession each of four species of *Poa*:

P. canbyi	43 highly resistant		
P. cur	ta 51 highly resistant		
P.	palustris 65 highly resistant	Culture	22
P.	palustris 65 moderately suscep	tible Culture	20
P. cur	ta 51 very susceptible		
Р.	pratensis 69 highly resistant	Culture	21
	pratensis 69 moderately suscep		
P. canbyi	43 very susceptible	Culture	14

DISCUSSION

Each of the 5 cultures studied was pathogenically distinct, so that this work is the first definite record of pathogenic specialization within Erysiphe graminis from Poa. Three different mildew races occurred on P. pratensis, and there are undoubtedly more yet to be isolated. Thus, strains of P. pratensis resistant to mildew in one area very likely will be infected in other areas where different races of E. graminis probably occur or in the same area upon the introduction of new mildew races.

Cultures 1, 21, and 22 from *P. pratensis* infected primarily related species of the *Pratenses* group; culture 14 from *P. ampla* principally species among

the Scabrellae and Nevadenses groups; and culture 20 from P. palustris almost exclusively species of the Palustres group of the genus Poa. This information will aid not only in understanding the host range and possible sources of inoculum of different races, but it also affects the evaluation of artificial and field tests of grass strains for mildew resistance. Mildew from P. ampla or P. palustris is probably of little consequence to the mildew problem in P. pratensis, since P. pratensis strains appear to be naturally resistant to those groups of mildew races. Likewise mildew races from P. pratensis appear not to be the ones responsible for damage to P. ampla, P. palustris, and related species.

Poa ampla, P. palustris, and P. pratensis and their relatives are growing in areas, especially the Pacific Northwest, where abundant perithecia form on certain species of Poa and where hybridization between races of mildew and segregations for pathogenicity probably are occurring. There is probably a natural selection of new races, screened out by the available host plants. Separation of Poa mildews into groups of races each of which infects a group of related grass species may be expected to parallel the groups of dominant species of Poa available as host plants. It is logical that mildew races that infect Poa pratensis should find closely related host species much more congenial than distantly related species. However, this does not exclude the possibility of genetic combinations within the fungus that will enable a mildew race to infect many species in several subgeneric groups of the genus Poa.

The mildews from Poa are not necessarily restricted to species of this genus. Several grasses can serve as common hosts for two or more different Poa mildew races and furnish a "breeding court" for hybridization between widely different races. Puccinellia distans 251 was very susceptible to both culture 14 from Poa ampla and culture 21 from P. pratensis. The opportunity for hybridization between different pathogenic races may be much reduced on certain grasses such as Poa pratensis and P. palustris since perithecia are seldom found on these species. Perithecia do form in abundance on P. ampla, P. canbyi, P. juncifolia and related species.

Two instances of natural mixtures of several mildew races differing greatly in pathogenicity on the same grass plants and many instances where several races could occur on the same grass were described in a previous paper (1). In Erysiphe graminis the production of new pathogenic races probably is limited only by the possible genetic combinations and by the sexual reproduction processes. Nothing has been reported concerning the nature of sexuality in E. graminis, but regardless of the exact nature of the processes involved, it is obvious that very pronounced segregations for pathogenicity have taken place.

Collections within species of *Poa* vary considerably in reaction to powdery mildew, a fact of importance in the selection and breeding of strains for mildew resistance. Bluegrass collections resistant to all five mildew cultures are: *Poa ampla*, 38, 42, 55, *P. canbyi* 44, and *P. juncifolia* 56. Mildew resistant

tance in these accessions is of considerable importance to agriculture in the Pacific Northwest where these bluegrass species constitute an important component of the native forage and where powdery mildew is a destructive disease on these species. *P. pratensis* 66 was highly resistant to cultures 1, 14, and 22, very resistant to culture 20, and moderately resistant to culture 21. It may be a desirable source of powdery mildew resistance, especially if it is resistant to other diseases.

In the genus Poa several very different modes of reproduction are known, and improvement methods are adjusted to meet the problems in individual species. Strains of P. pratensis have been isolated which are high-yielding when free of disease but which are susceptible to one or more destructive diseases. Improvement of such strains might be accomplished in several ways. Plants arising from occasional fertilization in apomictic strains and the possible segregations from both selfed and open-pollinated fertile strains might provide genetic recombinations resistant to one or more diseases to which the parents are susceptible. The high degree of apomictic reproduction in Poa pratensis has discouraged hybridization, and individual plant selection methods are largely utilized. However, successful hybrids between Texas bluegrass (P. arachnifera) and Kentucky bluegrass (P. pratensis) have been reported (7, pp. 1060, 1090) which compare favorably, especially in dry years, with outstanding selections of P. pratensis.4 This cross was made primarily to produce a drought-resistant bluegrass. P. arachnifera, in addition to drought resistance, also carries resistance to certain diseases. One accession has been resistant to certain cultures of Erysiphe graminis, Puccinia poae-sudeticae, and Helminthosporium vagans. Therefore, it appears that the possibilities with this cross have not been fully exploited. The development of an outstanding strain of P. arachnifera should increase the value of hybrids with P. pratensis. If the hybrids should have disease resistance in addition to the agronomically desirable characters of the parent P. pratensis it would be an excellent method of overcoming susceptibility to disease in otherwise outstanding strains of P. pratensis. The fact that part of the hybrids are fertile suggests that this cross may greatly alleviate disadvantages residing with apomictic strains of P. pratensis. Fertility will permit the utilization of advantageous breeding methods that are not applicable to improvement of apomictic strains.

Striking resistance to all the cultures of powdery mildew was found in certain accessions of *Poa nemoralis* and *P. sphondyloides*. Preliminary studies also indicate that these species constitute a source of resistance to other diseases as well.

SUMMARY

Selected groups of 398 accessions of 116 species and 8 varieties of grasses in 28 genera were inoculated with five cultures of *Erysiphe graminis* obtained from three species of *Poa*.

4 From Mr. E. Marion Brown, in correspondence.

Each of the five cultures is pathogenically distinct; thus, pathogenic specialization is recorded for the first time in E. graminis from Poa. Three of the cultures infected grasses outside the genus Poa, although each culture infected principally Poa species in the subgeneric group embracing the species on which it was obtained. Three different pathogenic races of powdery mildew were isolated from P. pratensis from three widely separated localities.

Several economic as well as wild species of Poa have been notably resistant to all five cultures.

Two advantages of hybridization between Poa arachnifera and P. pratensis in improvement of Kentucky bluegrass are (1) the possibility of obtaining disease resistance from P. arachnifera, and (2) the production of fertile hybrids from apomictic P. pratensis parents, thus permitting utilization of plant breeding methods not applicable to improvement of apomictic strains.

KENTUCKY AGRICULTURAL EXPERIMENT STATION. LEXINGTON, KENTUCKY.

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HYPOXYLON PRUINATUM AND ITS PATHOGENESIS ON POPLAR¹

R. H. GRUENHAGEN²

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INTRODUCTION

The diseases of poplar have come into prominence because of the recently recognized value of poplar3 in the forest economy of the North Central Region of the United States. The wood of these trees is used extensively for boxwood, paper pulp, and excelsior. The trees are valuable in forestry as a green-leaf firebreak planting, as a nurse crop for conifers, and as a source of deciduous leaves to help maintain soil fertility (15). No other trees seem so well suited for fulfilling such needs. A continuous supply of poplars can be maintained because they are fast-growing trees, are well adapted to much land that is submarginal for agriculture, are light-seeded and come in rapidly after a forest fire, and in many cases can be propagated vegetatively. Certain diseases, however, tend to offset these advantages.

The poplar canker, caused by Hypoxylon pruinatum (Kl.) Cke., was first described in the United States by Povah (10) and in Wisconsin by Kaufert (6). Its prevalence in Michigan, New York, Maine, and Ontario, Canada, has been reported by other workers (1, 10, 13). The incidence of the canker in several national forests in the Lake and Central States was studied by Lorenz and Christensen (8), who indicate that the canker is the most important poplar disease in the Lake States.

The value of the host and the importance of the disease have warranted further study of the canker, the results of which are recorded in this paper.

OCCURRENCE OF THE DISEASE

The prevalence of Hypoxylon canker on poplar in Wisconsin was studied during the summers of 1941, 1942, and 1943. Twenty-six field study plots, each 1 acre in size, were established in 8 counties: 4 plots in Bayfield, 2 in Douglas, 4 in Iron, 2 in Marinette, 5 in Oneida, 4 in Vilas, 3 in Washburn, and 2 in Waushara. Every poplar in each plot was tallied by diameter class and was examined for Hypoxylon canker. The canker infection ranged from 0 per cent to 53 per cent with an average of 24 per cent. The site index (height of the average dominant tree at 50 years of age) for each plot was

1 Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

² Formerly research assistant, Wisconsin Agricultural Experiment Station. Acknowledgment is made to the Wisconsin Conservation Department for its cooperation and encouragement in this investigation. The writer is indebted to C. M. Christensen, Carl Hartley, Frank Kaufert, and C. A. Richards for advice concerning various phases of the work; to A. J. Riker for help and encouragement during the course of this study; and to Eugene Herrling for assistance in preparing the illustrations.

3 Unless otherwise stated, the term "poplar" in this paper refers to Populus tremu-

loides Michx. and to P. grandidentata Michx.

determined by the method described by Kittredge and Gevorkiantz (7). The summaries of the cruise records are in tables 1 and 2.

In these data the prevalence of the disease was highest on the poorest site surveyed (Table 1, site index 40) and was lowest on the best site surveyed (Table 1, site index 80). This confirms earlier reports. While the reason for this was not determined, it is suggested that on the good sites the rate of escape, the disappearance of infected trees, and the resistance of the trees were greater than on the poor sites.

TABLE 1.—Incidence of infection with Hypoxylon pruinatum by site index classes²

Site index class	Trees examined	Trees with Hypoxylon canker			
40 50 60 70 80	Number 168 112 716 408 178	Per cent 51 24 22 22 4			

^a A portion of these figures is used through the courtesy of Dr. E. E. Honey, Emergency Plant Disease Prevention Project.

TABLE 2.—Incidence of infection with Hypoxylon pruinatum by tree size (d.b.h.)a

D.b.h.b	Trees examined	Trees with Hypoxylon canker				
$\begin{array}{c} Inches \\ 0-1 \\ 1-2 \\ 2-3 \\ 3-4 \\ 4-5 \\ 5-6 \end{array}$	Number 253 153 222 218 173 173	Per cent 5 27 31 44 33 31				
6-7 7-8 8-9 9-10 10-11	125 79 32 18 9	28 29 12 11 0				

a A portion of these figures is used through the courtesy of Dr. E. E. Honey, Emergency Plant Disease Prevention Project.

b Diameter at breast height or 4.5 feet.

There was a high prevalence of disease on trees that were less than 8 inches d.b.h. (Table 2). The factors that governed disease prevalence on different sites probably also accounted for this fact. On the larger trees cankers were usually restricted to the branches or to the small upper part of the trunk, which is commercially unimportant.

HOSTS INFECTED, THEIR RANGE, AND CANKER DISTRIBUTION

The same Hypoxylon canker is found most frequently on Populus tremuloides and P. grandidentata, but it occurs to a lesser extent on P. balsamifera. Other species of poplar are also attacked by different species of Hypoxylon. P. nigra var. italica Du Roi is attacked by H. malleolus B. and Rav. and P. trichocarpa T. and G. by H. serpens (P.) ex Fr. (14).

The species of Hypoxylon are not restricted to poplar, however. While the complete host range has not been established, H. pruinatum has been found producing injury on Betula; H. morsei on Alnus; and H. blakei on species of Acer, Quercus, and Salix (1). The work to date (1) indicates that one or another species of Hypoxylon may be found on most of the hardwood species.

The natural distribution of *Populus tremuloides* and *P. grandidentata*, the most frequently involved hardwoods, is reported by Sargent (12) and Munns (9). The Hypoxylon canker has not yet been reported as occurring throughout the complete host range. Its wide distribution has been recorded by several workers (1, 3, 4, 6, 8, 10). Wherever it was found, the disease had similar characteristics.

SYMPTOMS

Poplar stands infected with Hypoxylon canker stood out in marked contrast to healthy stands. Characteristic features observed were the broken trees, the dead tree stubs, and the blackened areas on the green trunks (Fig. 1, A). Cankers that girdled branches produced brown "flags" throughout the stand. If the branches beyond the canker were not dead, the leaves were usually smaller and pale green to yellow.

Incipient cankers appeared as yellow to orange, slightly sunken areas in the bark. The margins were usually irregular and lobed. On older infections blister-like conidial fructifications and erumpent perithecial stromata were clearly visible.

THE CAUSAL ORGANISM

Morphology

The morphology of $Hypoxylon\ pruinatum$ has been discussed by Bier (1), so the details are omitted in this paper. A stroma of the imperfect stage is shown in figure 1, B, and of the perfect stage in figure 1, C. Mycelial fans and host discoloration are shown in figure 1, D. Asci, ascus stalks, and ascospores are illustrated by Bier (1).

Separate measurements were made of four perithecial collections from Madison and four from Trout Lake, Wisconsin, to determine the average size of the spore-bearing part of the ascus, of the ascus stalk, and of the ascospores. Measurements of perithecial contents mounted in water were taken with an ocular micrometer on 50 spores and 25 asci from each collection. The results, averaged by localities, are in table 3.

The spore measurements for the 8 collections agree closely with those made by Bier (1). There are some deviations in ascus measurements that may be explained by differences in the development of the asci.

The attendant taxonomy has been discussed by Bier (1). It has been confirmed and has been accepted by the present writer. There was no

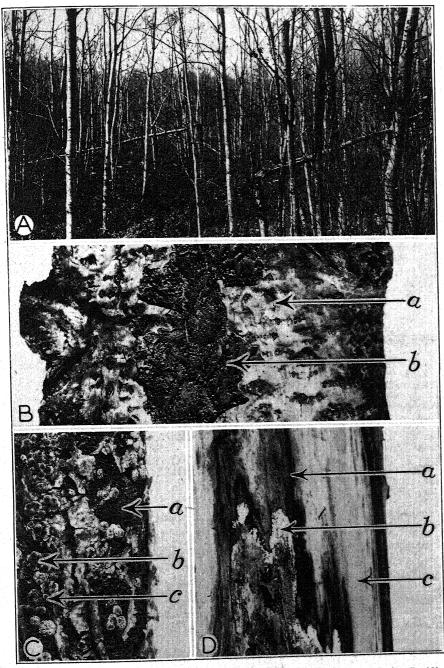


Fig. 1. A. Cankered poplar stand in Lincoln County, Wisconsin. B. Conidial stroma showing (a) "blister" of the outer bark and (b) "mycelial pegs" where outer bark has been peeled away. C. Fruiting canker showing (a) old conidial stroma, (b) perithecial stroma, and (c) ostioles of perithecia. D. Portion of canker with the bark removed to show (a) discoloration preceding mycelium, (b) mycelial fan, and (c) healthy wood.

significant change in morphology when the fungus was grown under several different circumstances.

Influence of Temperature, pH, and Certain Nutrients on Fungus Growth

The influence of temperature was investigated on the rate of growth of Hypoxylon pruinatum in culture on 2 per cent malt agar. Petri dishes were poured with 20 cc. each of medium adjusted to pH 6.5. Each plate was seeded with a 6-mm. disk of inoculum from a 10-day-old culture on 2 per cent malt agar. This 6 mm. was subtracted from the final growth readings. Five replications were incubated at 9 temperature levels in 4° C. steps from 4° to 36° C. Diameters were measured every other day for 8 days.

The results were averaged for 3 trials each with 5 replications on 4 single-spore isolates. The most rapid diameter growth was at 28° C. There

TABLE 3.—Comparison of ascus and ascospore size of two Wisconsin collections and one Canadian collection

	Material collected near								
	Madison, Wis- consina	Trout Lake, Wisconsina	Ottawa, Ontario						
Spore-bearing part of	Microns	Microns	Microns						
ascus	128 to 176 32 to 80	152 to 187 32 to 48	160 to 190 40 to 60						
Ascospores, average	22 to 29 by 8 to 13 26 by 10	22 to 29 by 8 to 13 25 by 10	24 to 30 by 9 to 13 26 by 11						

a Collected and measured by the writer.

was no growth in 8 days at 4°, 8°, or 36° C. The data are omitted because they are so similar to those based on weight.

Temperature also influenced the weight of mycelium produced by *H. pruinatum* in 2 per cent malt extract in distilled water. The reaction of the medium was adjusted to pH 6.5. Erlenmeyer flasks of 250 cc. capacity, each containing 150 cc. of the sterile, adjusted medium, were each seeded with a 6-mm. disk of inoculum. Five replications were then placed for 16 days in incubators at temperatures from 4° to 36° C. At the end of 16 days the mycelial mats which developed in the flasks were separated from the culture medium and were washed, dried, and weighed. The weights for 3 trials of 5 replications each were recorded and averaged for each temperature group (Fig. 2).

The greatest weight of mycelium of *H. pruinatum* was developed at 28° C. There was good growth in the range from 20° to 28° C. and no growth at 4°, 8°, or 36° C.

The importance of acidity (pH) also received attention in relation to the weight of mycelium produced by *H. pruinatum*. Malt-extract liquid

b Collected and measured by J. E. Bier (1).

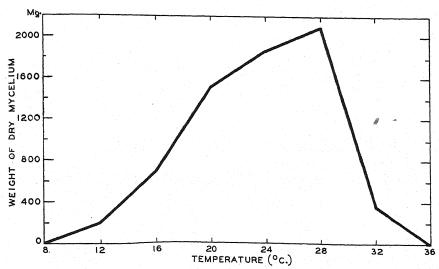


Fig. 2. The influence of temperature on growth of H. pruinatum in liquid culture.

medium in 250-cc. Erlenmeyer flasks was buffered with K₂HPO₄ (5 g. per liter in the alkaline range) and KH₂PO₄ (5 g. per liter in the acid range). The reaction of the medium was adjusted aseptically before inoculation according to the intervals shown in figure 3. There were 5 replications at each point of adjustment. After 16 days' incubation at 28° C. the mycelial mats were washed, dried, and weighed. The pH, read at the start and at the end of the trial, did not drift more than 0.1 pH unit in any flask during the 16 days.

The weights were averaged by pH groups. The average results of a typical trial of 5 replications are in figure 3. The greatest mycelial weight

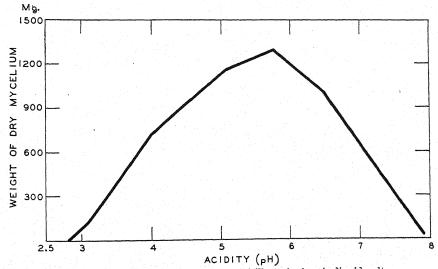


Fig. 3. The influence of acidity on growth of H. pruinatum in liquid culture.

of *H. pruinatum* was produced at pH 5.6. Satisfactory growth developed between pH 4.5 and 6.5.

The effect of 8 various culture media on rate of diameter growth and rate of conidial production appeared in 4 trials of 4 replications each. Six of the media used were malt agar, potato-dextrose agar, onion agar, Limabean agar, pea agar, and water agar. All were made according to Riker and Riker (11) and were adjusted to pH 6.5. Healthy poplar bark was ground in a Wiley Mill No. 1, was cooked in distilled water (50 g. of tissue per liter) for 1 hour, and made into 2 per cent agar. Half of the medium was made with strained tissue decoction and half with unstrained. The reaction was adjusted to pH 6.5. The same procedure was followed with Hypoxylon-infected poplar bark. Petri dishes were poured, seeded, and incubated at 28° C. for 21 days. Records on diameter growth and production of conidia were taken every other day.

The averages of 4 trials of 4 replications each showed: (1) that the cultures grew most rapidly on malt agar, filling the dishes (92 mm.) in 10 days, and slowest on cankered bark decoction agar; (2) that those on unstrained healthy bark decoction filled the dishes in 17 days; (3) that conidia were produced by cultures on malt agar in 5 days, on onion agar in 11 days, and on potato-dextrose agar in 13 days; and (4) that no conidia were produced on the other substrata within 21 days.

The investigations of the fungus as it developed in vitro were continued with an examination of its host relations.

ENTRANCE OF THE FUNGUS INTO THE HOST

Field inoculation studies were conducted during the summers of 1942 and 1943 to determine the means by which *Hypoxylon pruinatum* entered the host. The following types of inoculum were used: (1) mycelial cultures on 2 per cent malt agar, (2) conidial suspensions in distilled water, (3) ascospore suspensions in distilled water, and (4) ground-up masses of diseased tissue, fruiting structures, conidia, and ascospores. The conidia, ascospores, and diseased tissue were all collected from natural infections in the field.

Several types of inoculation were tried. In all cases, the tissue to be treated was first wiped over with a cloth dampened with 70 per cent alcohol and allowed to dry.

Unwounded tissue was exposed by the application (1) of 1 cm. square blocks of culture and agar, (2) of sprayed spore suspensions, and (3) of diseased-tissue inoculum. Regardless of the type of inoculum used, half of the treatments were left uncovered. One-fourth were sealed with adhesive tape, and one-fourth were wrapped with wet cotton and wax paper as described by Bier (1).

Cuts for wound inoculations were made in several ways. Cuts in an inverted "V" or "U" shape 2 to 3 cm. high by 1 to 2 cm. wide were frequently used. Small bark slits 3 to 7 cm. long, as well as bark punctures, were also used. Different wounds, respectively, were covered or left uncovered.

Some tissue was bruised and wounded before inoculation. Trunk tissue, approximately 25 to 50 sq. cm., was crushed by striking it several times with the blunt end of a hand ax. Two types of inoculation wounds were used: (1) a horizontal cut made through the bark and into the cambium near the center of the bruised area with the sharp edge of the ax, and (2) a puncture 4 to 6 mm. in diameter through the bark at the center of the bruised area. Either spore suspensions or ground-up diseased tissues were used for inoculum.

Insect borer holes were inoculated by packing into them a paste made of ground-up diseased tissue and spores in water. Half the holes were left open and half were covered with wet cloth.

Buds, leaves, petioles, and leaf axils were inoculated in both wounded and unwounded condition respectively with spore suspensions and mycelial cultures. Wounds were made with a dagger-scalpel. No attempt was made to surface sterilize the buds, leaves, petioles, or leaf axils. Spore suspensions were sprayed on, and the mycelial cultures were applied in 5-mm.-square blocks of culture and agar.

The results of the inoculations are summarized in table 4. All inoculations and controls on buds, leaves, petioles, and leaf axils, whether wounded or not, gave negative results. All inoculations and controls on unwounded branch and trunk tissues remained uninfected, as found by Bier (1). Inoculations and controls on branch stubs and in insect borer holes also remained uninfected, but it is possible that the infection court had been closed by wound tissue before inoculation. The inoculations on cut branch and trunk tissue were from 4 to 60 per cent positive. All covered controls were uninfected, but 7 per cent of the open controls became infected. The infection of the open controls was doubtless caused by natural disease spread from fruiting cankers in the vicinity. All inoculations on bruised tissue were positive. All covered controls remained uninfected. The open controls were 60 and 50 per cent infected, respectively, for ax wounds and for puncture wounds on bruised areas. Natural spread from fruiting cankers was again held responsible. The data indicate that H. pruinatum is a wound parasite on bark tissue.

Several types of branch and trunk wounds were found during field observations in Wisconsin during the summers of 1941, 1942, and 1943 that could act as infection courts in natural poplar stands. Some of these infection courts, all of which had been found at one time or another at the centers of cankers, were caused (1) by weather, e.g., wind, ice, lightning, (2) by man, e.g., ax wounds, logging damage, (3) by animals and birds, e.g., deer scraping, porcupine chewings, sap-sucker borings, and (4) by insects, e.g., bark borers, chiefly Saperda calcarata Say. A supplementary study was made on the relation of insect-borer holes to incidence of new cankers.

All of the insect-borer holes up to 8 feet above the ground were examined in all the poplars on 11 plots, each covering $\frac{1}{10}$ acre, located in Dane, Vilas, and Waushara Counties in Wisconsin. Of the 1018 borer holes examined,

TABLE 4.—Results of the 1942 and 1943 field inoculations with Hypoxylon pruinatum

				Urmoralon
Kind and place of inoculation	Kind of inoculum	Covered or open	Treatments	cankers
Young growth			Number	Per cent
Buds, leaves, petioles, and leaf axils; wounded and not wounded	conidial and ascosporic suspensions, myeelial cultures, controls	{ half of each covered, } { rest open }	1600	0
Older growth Branch and trunk	op		CCC	
Unwounded	mass inoculum		200	• • • • • • • • • • • • • • • • • • •
	(conidial suspensions	(covered	40	37
Slit. inverted ((II') or ((V))	ascosporie suspensions	open covered covered covered	40 40 40	50 60
}	mycelial culture	covered (40	46
	[control	covered open	$\frac{120}{210}$	0 2
Bark puncture	{ mass inoculum } control	covered	25 25	4 0
	mass inoculum	covered popen	40 40	100
Brused and ax cut	[control	covered open	40	09
	mass inoculum	f covered open	20 02 03	100
Bruised and puncture	control	covered open	02 G 03 G	50
Insect borer holes	mass inoculum and controls	{ half of each covered, } { rest open }	100	0
Dead branch stubs	(mass inoculum) control	open	22 25 55	00

14, or 1.4 per cent, were at the center of cankers. Since so few of the borer holes were infected, the borer itself did not seem to be an active agent in the dissemination of the disease. However, it was obvious that the borer holes were open infection courts and, because of their great numbers in the woods, had to be considered in the infection complex.

From insect injuries and other wounds a dark, sticky liquid frequently was exuded, which, during dry weather, hardened into a "varnish." Some of this exudate was incorporated into water-agar medium and seeded with a mycelial culture of $Hypoxylon\ prwinatum$. Water-agar controls were used. Two trials of 5 replications each were incubated at 28° C. for 16 days. The resulting cultures on water-agar plus "varnish" had an average diameter that was 40 per cent greater than that in the controls. This suggests that the sticky wound exudate might act not only as a spore catcher, but also as a

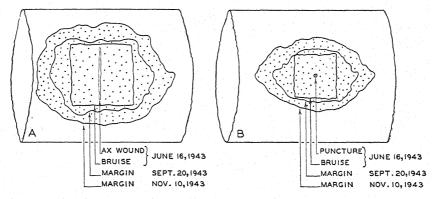


Fig. 4. Average advance of artificially induced cankers. A. Square bruised area with an ax wound, average of 24 cankers. B. Square bruised area with a puncture wound, average of 17 cankers.

culture medium when suitably wet. The entire insect relations problem deserves further study.

The development of lesions following inoculation was recorded for 200 different trees. The first symptoms appeared in 2 to 8 weeks. The rate of canker spread, which was most rapid up and down the tree, was observed on 41 cankers from 2 types of wounds shown diagrammatically in figure 4. The measurements were based on the surface appearance and independent from the spread of the fungus within the tissues.

DEPTH OF FUNGUS PENETRATION

When the bark was removed from active Hypoxylon cankers, mycelial fans frequently were found growing over the surface of the wood (Fig. 1, D). To determine how deeply the fungus penetrated into the host tissues, radial sections of active cankers were made on a sliding microtome. A differential stain was secured with the methyl violet—Bismark brown technique described by Hubert (5). Microscopic examination of the sections showed mycelium in the bark, the cambium, and the outer 4 to 5 mm. of wood tissue. These

observations were checked by cultural studies, in which cankered tissues outside of the cambium were removed with sterile scalpels to prevent contamination of the inner tissues. Chips of wood were taken from different depths in the wood tissue and were transferred aseptically to 2 per cent malt agar in Petri dishes. Isolations were made also from the bark and the cambium. Incubation was at 28° C. for a maximum of 16 days. The data (Table 5) indicate that the organism is located in the host bark, cambium, and outer 8 to 9 mm. of the wood, where it apparently had an opportunity to live from one season to another.

TABLE 5.—Results of attempts to isolate Hypoxylon pruinatum from different depths in host tissue (Populus tremuloides)

Tissue and depth cultured	Specimens	Isolations	Isolations yielding Hypoxylon
Bark Cambium Wood 0.5 mm. below cambium 1.0 do 3.0 do 5.0 do 8.0 do 10.0 do	Number 1 1 1 2 2 4 2 2 2	Number 48 48 48 96 96 192 100 100	Per cent 100 100 100 100 75 12 2 0

OVERWINTERING

The means of overwintering of the fungus was determined by isolation of 32 single ascospore and 50 mass-tissue cultures from 5 cankers that were collected every month from November to April. At least 60 per cent of the ascospores were viable, and 80 per cent of the tissue isolations yielded Hypoxylon every month. No viable conidia were found during this period. This indicated that the organism overwintered both as mycelium in infected tissue and as ascospores which were already available to spread the fungus the following season.

DISSEMINATION

The natural means of dissemination of Hypoxylon pruinatum was studied during the summers of 1942 and 1943. To determine the direction of natural spread in the field, the locations were plotted of all new and old infections on 4 one-tenth acre plots, 2 in Vilas County and 2 in Dane County, Wisconsin. The prevailing summer winds for these two areas were from the south and the southwest.⁴ Of the 250 new cankers examined, 80 per cent were down wind from and facing toward the old fruiting infections. This observation prompted experimentation on wind dissemination.

A spore trap was constructed that was similar to Maddox's "Aeroconiscope" (2). The trap was set up in the center of a poplar plot 15 feet from the nearest canker. The spore content of the trap was examined every week

4 Courtesy of Eric Miller, U. S. Weather Bureau, Madison, Wisconsin.

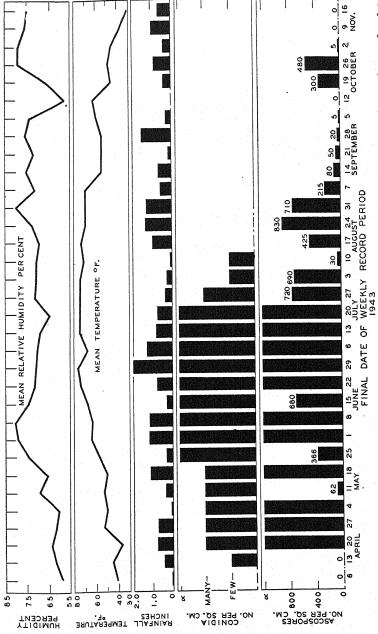


Fig. 5. Mean relative humidity, mean temperature, rainfall, and spore trap counts in Hypoxylon canker study plot, Madison, Wisconsin, 1943.

from April to the middle of November. The results of the counts and the accompanying weather records are in figure 5. Conidia or ascospores, or both, were present in the air from the second week in April through October, with the exception of the second week in October when there was no rain. This indicates that wind dissemination was in progress throughout the entire period.

Further evidence on wind dissemination came from infection counts on trees that were bruised and wounded (see table 4). Sixty wounds were covered with adhesive tape and 60 were left open. By the end of 5 months, 57 per cent of the open wounds were infected with *H. pruinatum*, while all the covered wounds were uninfected. This could be explained best on the basis of wind-borne spores.

An attempt was made to study the natural incidence of disease in relation to time of year, meteorological conditions, and availability of inoculum. However, the incidence of disease each week, which was less than half of one per cent, gave figures that were too small for satisfaction. To secure significant figures would have involved frequent observations on thousands of tagged trees. Since it was not feasible to examine such numbers, attention was directed toward the influence of weather on the availability of inoculum.

ENVIRONMENTAL CONDITIONS INFLUENCING CONIDIAL DETACHMENT AND ASCOSPORE DISCHARGE

The influence of time of year and of environmental conditions favoring and retarding conidial detachment and ascospore discharge was investigated during the summers of 1942 and 1943. Greased microscope-slide spore traps similar to those described by Bier (1) were placed on cankered trees so that the incidence and intensity of spore release could be observed. The greased slides over 4 cankers showing the perfect stage of Hypoxylon pruinatum were placed 4 mm. away from the perithecial stromata. Those on the 2 conidial cankers were placed facing the prevaling winds. Spore counts were made on all the slides every week from April to the middle of November. The number of ascospores was counted up to 900 spores per sq. cm. but beyond that was given an infinity notation. The conidia were small and hyaline, so their number was estimated. The value "Few" equalled 0 to approximately 100, "Many" equalled approximately 100 to 500, and "\infty" equalled over 500 spores per sq. cm. The count of the spore load in the "Aeroconiscope" spore trap is in figure 5.

Conidia first appeared in the trap the second week in April. The load increased to a maximum by the fourth week in May and held a constant high level until the third week in July when it started to drop off to an end the third week in August.

A comparison of the trap record with temperatures and humidities for the period suggests that the increase from April to June in the numbers of conidia dispersed was correlated with the increase in mean temperatures and

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mean relative humidities during the same period. There appeared to be no correlation with rainfall.

To determine whether free water was necessary to conidial detachment, greased glass slides were fastened 1 cm. away from 5 cankers showing the imperfect stage. Conidial counts were made on the slides for 10 one-hour periods during 10 days that were rainless. This was repeated for 10 rainy days. A representative average showed that 234 conidia and 182 conidia were caught per hour per sq. cm. of glass slide during dry periods and rainy periods, respectively. The trend of these observations was confirmed repeatedly under controlled laboratory conditions. This indicated that free water was not necessary for conidial detachment.

The first ascospores were found in the trap during the third week in April and the last ones were found on the second of November. The two end points seem to be correlated with a mean temperature below 45° F. The low mean temperature for the week ending April 20 was caused by two cold days, April 14 and 15. The heavy spore discharge for that week took place on April 17, 18, and 19. The high mean summer temperatures did not seem to influence ascospore discharge. The mean relative humidity, except when it indicated the absence of rain, e.g., week ending October 12, had no influence on the incidence of ascospore discharge. There was a rather close correlation between the amount of rain and the volume of spore discharge.

The ascospore release was recorded at 2-hour intervals in the field from 10 p.m. June 14 to 10 a.m. June 16, 1943. Spore counts were made on 4 greased glass slide traps. The average results of the spore counts and the corresponding weather data are in figure 6.

There was no significant relation between temperature and amount of ascospore discharge for the 36 hours of the trial. There was an apparent relation between relative humidity and ascospore discharge. Closer examination of the data reveals, however, that the periods of rain, which influenced the relative humidity, were more likely the critical factors in spore discharge. This is illustrated by the records taken at 6 a.m. June 15. At that point, between rains, the relative humidity was still approximately 100 per cent, but the spore discharge had fallen to 700 spores per sq. cm. The spore discharge fell to 50 spores per sq. cm. at 10 a.m., approximately 2 hours after the 6:10 to 7:30 shower, even though the relative humidity was still approximately 100 per cent in the woods. This suggests that free water is necessary for the initiation of ascospore discharge.

A few ascospores were caught in the traps as long as 20 hours after a rain. It may be that ascospores caught in drops of water during the rain clung to the tree and were blown off long after cessation of ascospore discharge from the perithecia; that ascospores that had fallen to the ground were redistributed by air currents; or that ascospores floating in the air for a long time finally came to rest on the slides.

The necessity of free water in initiating ascospore discharge was studied in the laboratory. A chamber (Fig. 7) was constructed in which the relative

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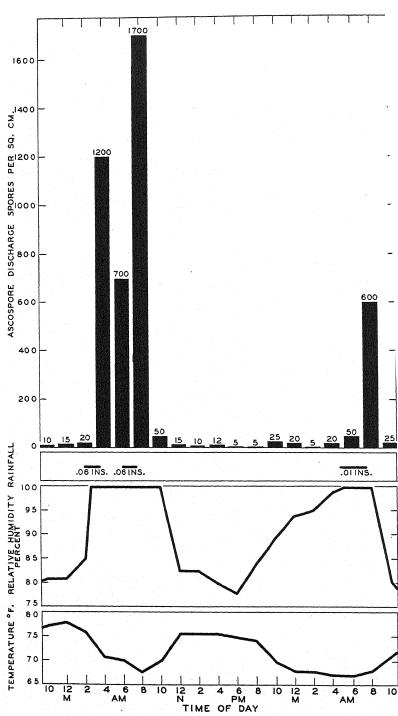


Fig. 6. Temperature, rainfall, relative humidity, and ascospore discharge Hypoxylon canker study plot, Madison, Wisconsin, June 14 to 16, 1943.

humidity was regulated by a stream of water-saturated air. Water was atomized into a 5-gal. carboy. The water-laden air from there was run through a water trap to remove any free moisture and from there into the chamber. The relative humidity in the chamber was calculated from wet and dry bulb thermometer readings.

Air-dry perithecial stromata were placed in the chamber and kept for 21 days in an atmosphere without free water. No ascospores were discharged. The relative humidity approached saturation, and so was comparable to that in the woods during a rain. This was repeated 3 times each with different perithecial stromata but with the same result. When perithecial stromata from the same source were soaked in water for one-half hour before being placed in the chamber, ascospore discharge was recorded as early as one hour after introduction into the chamber and averaged 407

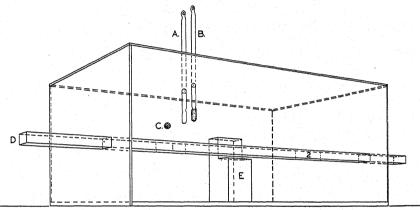


Fig. 7. Ascospore discharge chamber with front face removed. A and B. Dry and wet bulb thermometers. C. Inlet hole for water-saturated air. D. Sliding bar carrying greased glass slides 1 and 2 in such a way that slides could be removed and replaced without opening chamber. E. Standard on which perithecial stromata were fastened so as to face the greased slide and allow 4 mm. between ostioles and slide.

spores per sq. cm. per hour for a 3-hour period in a relative humidity approaching saturation. Thus, free water was necessary for the initiation of ascospore discharge.

DISCUSSION

Various lines of work, reported by earlier writers (1, 6, 8, 10) about Hypoxylon canker on poplar, have been repeated and confirmed for Wisconsin, although the details have been largely omitted to save space. Among these items are the following: the economic importance of poplars, the destructiveness of Hypoxylon canker, and the morphology and attendant taxonomy of the causal organism.

The means by which this fungus operates both in the laboratory and in the woods have been elucidated. The study of the life history in relation to pathogenesis has shown how the fungus grows and sporulates at different times of year and under varying conditions of weather. The relatively low -10

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percentages of positive results suggest that this fungus infects with some difficulty through wounds made by cutting. However, if it can start in association with bruised and killed tissue, infection occurs easily and practically every time. A practical consideration is the development of an inoculation technique whereby one can be confident of a high percentage of infection from artificial introduction of spores. This is a critical item for making quality tests of poplar selections.

The development of rapidly growing poplar hybrids, which are capable of rooting from cuttings, has attracted considerable attention in recent years. However, if such hybrids should prove as susceptible to Hypoxylon canker as many of the native poplars, plantations of them will certainly lead to serious disappointment. By means of the technique now made available, tests of resistance by these and other selections to Hypoxylon canker can be employed with confidence, and the susceptible selections can be eliminated.

Meantime, from the several lines of evidence presented, it is clear that Hypoxylon cankers in the woods are a continual source of inoculum for any healthy trees in the neighborhood. Consequently, if the value of the stand justifies the expense, the trees affected with Hypoxylon canker should be removed and burned.

SUMMARY

A study was made of *Hypoxylon pruinatum*, of its life history in relation to pathogenesis on poplar, and of the associated Hypoxylon canker which is the most important disease on poplar in the Lake States.

The Hypoxylon canker was found on an average of 24 per cent of all the poplars examined in Wisconsin and the corresponding symptoms are described.

Surveys showed that there was more canker on poor growing sites than on good sites.

The causal organism made the best diameter growth in culture and developed the greatest mass of mycelium at 28° C. The optimum hydrogen-ion concentration range for growth in culture was from pH 4.5 to 6.5. The fastest rate of growth and rate of conidial production observed was on 2 per cent malt agar.

The organism is a wound parasite on branches and trunks, but buds, leaves, petioles, and leaf axils were not infected. Only a moderate percentage of infection developed from inoculation wounds made by cutting. However, one hundred per cent infection was secured when the trunk and branch bark was bruised and wounded before inoculation. Examination of the cankers revealed the organism in the bark, cambium, and no deeper than 8 mm. into the wood.

The organism overwintered in host tissue as mycelium and ascospores and was disseminated by wind.

Conidia were detached under either wet or dry conditions, but free water was necessary for initiating ascospore discharge. There was no ascospore

discharge in the early spring or the late fall when the mean temperature was below 45° F.

By means of inoculations through bruised tissue a method has been developed for testing the relative resistance of various poplar selections.

UNIVERSITY OF WISCONSIN.

MADISON, WISCONSIN.

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ROOT DEFECTS AND FUNGI ASSOCIATED WITH THE LITTLE-LEAF DISEASE OF SOUTHERN PINES

L. W. R. JACKSON1

(Accepted for publication September 20, 1944)

INTRODUCTION

A serious deterioration and dying of southern pines, particularly short-leaf pine (*Pinus echinata* Mill.), known as the little-leaf disease, was first reported from Alabama in 1935. Extensive surveys (11, 12) have shown that little leaf is widely distributed through much of the Piedmont region from central Virginia to western Alabama, and through the upper coastal plain of Alabama to northeastern Mississippi. The disease occurs extensively on shortleaf pine, and to a lesser extent on loblolly pine (*P. taeda* L.), Virginia pine (*P. virginiana* Mill.), and pitch pine (*P. rigida* Mill.), and symptoms resembling little leaf have been found occasionally on longleaf pine (*P. palustris* Mill.). Typical little leaf has seldom been found on trees younger than 20 years or less than 3 inches in diameter at breast height.

The symptoms on the aboveground parts of affected trees are reduced needle length, varying degrees of yellowing, and a shortening of the twig internodes, which give the crown a sparse, tufted appearance.² When branch dying occurs, it usually progresses upward from the base of the crown. Another characteristic of little-leaf trees is a marked slowing down of diameter growth. Outer rings are often greatly reduced in width (2, 12). No infections related to little leaf and caused by pathogenic organisms have been found on the aboveground parts of diseased trees.

The root systems of shortleaf pines affected with little leaf are consistently more defective than those of healthy trees. There is an excessive exfoliation of bark, producing brown patches, dieback of the fine roots, and large, pitchy, cankerlike lesions.

The cause of the little-leaf disease has not been determined. The work on the cause has been based chiefly on three hypotheses, namely, that little leaf results from (1) a root deterioration caused by pathogenic fungi, (2) a virus, or (3) physical and chemical soil factors. This paper presents work on the characteristics of the various root defects, the condition of the mycorrhizae, and the pathogenicity of two fungi found associated with root defects. Shortleaf pine was the only host used in the experimental tests.

 $^{^{1}\,\}mathrm{Associate}$ Pathologist, Division of Forest Pathology, United States Department of Agriculture.

² A detailed description of symptoms will appear in a forthcoming U. S. Dept. Agr. Circular entitled "Little-leaf disease of pine" by G. H. Hepting, T. S. Buchanan, and L. W. R. Jackson.

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ROOT DEFECTS

General Characteristics of the Root Systems of Diseased and Healthy Shortleaf Pines

The condition of roots of diseased and healthy trees was examined in detail by washing out the root systems of 3 diseased and 4 healthy trees with a high-pressure stream of water delivered by a force pump. Samples of the feeding roots3 were collected from each tree for an analysis of the amount of dieback and the condition of the mycorrhizae.

The root excavation work showed no observable difference in the distribution, extension, or gross morphology of the primary and larger lateral roots and the taproots of the diseased and healthy trees. On the diseased trees, the roots of all sizes, including feeding roots, were usually covered with a thick layer of brown patch. On the healthy trees, the large roots were covered with brown patch but the roots less than one inch in diameter usually had only small and widely scattered brown patches. Dead primary and lateral roots were common on little-leaf trees. Diseased trees had

TABLE 1.—Amount of dieback of feeding roots on diseased and healthy shortleaf pines

Trees and roots examined	Condition of trees		
rees and roots examined	Healthy	Diseased	
Total number of trees	4 775 2.4 17	3 679 2.8 71	

many more feeding roots that were dead or were dying back from the ends than had healthy trees.

The analysis of the condition of the feeding roots was based on samples of 139 to 281 feeding roots per tree from 4 healthy trees, and from 122 to 332 feeding roots per tree from the 3 diseased trees. Little-leaf trees had about 4 times as many killed-back feeding roots as did healthy trees (Table 1). In both diseased and healthy trees the feeding roots with dead ends had an average live length of 2 to 3 inches. These results, together with field observations, have shown that the bulk of the feeding roots on diseased trees are usually short and stubby, sometimes reduced to mere spurlike structures, and have from one to several lateral replacement roots. The dieback of feeding roots, such as occurs on a little-leaf tree, must greatly reduce the absorbing capacity of the root system.

The cause of the excessive dieback of feeding roots on little-leaf trees has not been determined. A certain amount of root decadence or dieback occurs normally on pines after the root system has fully developed. According to Büsgen and Münch (3), the lateral roots of healthy pines attain their

³ In this paper, the term feeding root is used for the small mycorrhiza-bearing lateral roots, which are usually less than one-tenth of an inch in diameter.

greatest spread in youth or up to about 14 years. They also state that the cessation of root elongation is accompanied by extensive dying of the ends of the roots and the replacement of the dead parts by lateral roots of a higher order. In working with pitch pine and shortleaf pine in New Jersey, McQuilkin (7) found that the stronger primary lateral roots ceased to elongate after the trees attained a height of 25 feet and a diameter of 4 inches. Although a certain amount of root decadence occurs normally on healthy adult pines, the dieback of the feeding roots on little-leaf trees is regarded as an important symptom. In working with Scotch pine (*Pinus sylvestris* L.), Laitakari (6) also found that some dead root ends occurred on vigorous trees, but the presence of many dead root ends indicated that the root system was retarded.

Condition of the Mycorrhizae

Root analysis showed no observable difference in either the condition or the abundance of mycorrhizae on diseased and healthy shortleaf pines. All of the mycorrhizae examined were richly branched and bifurcate, and grew as coralloid clusters. Mycorrhizae of diseased trees were always well developed and appeared normal if the feeding roots were healthy.

Characteristics of Brown Patch on Roots of Diseased and Healthy Pines

Histological study shows that the brown patches consist of older layers of the secondary phloem (Fig. 1, A), which have abscissed by the development of periderm layers. They are light brown when first cut off, but turn dark brown with age. The size and arrangement of the elements of the brown patches did not differ from those of the underlying live phloem, indicating that no pathological changes had occurred during their formation. Dark hyphae that closely resemble those of a *Torula* are abundant throughout the brown-patch layers.

Brown patches are irregular in outline and vary from minute spots to areas of several square inches. On healthy trees, the brown patches are widely scattered but they may encircle the root in localized areas. On severely diseased trees, however, most of the roots, including the feeding roots, are usually covered with a thick layer of brown patches, which give them a rough scabby appearance. Though variable in depth, these patches involve half or more of the phloem (Fig. 1, A). Brown patches are often much deeper on some of the poorer roots of little-leaf trees, sometimes extending to the current sieve-tube layer. In such extreme cases the secondary phloem is reduced to a thin white film, consisting of only the current sieve-tube layer. When pried out with a knife, the brown patches rupture along the periderm layer and leave a conspicuous depression in the bark.

An excessive exfoliation of root phloem, such as occurs in little-leaf trees, decreases the food-storage capacity of the root system. Microscopic examination of root and trunk bark of shortleaf pine collected in February

showed that brown-patch layers were always devoid of starch. In the living portion of the bark, the parenchyma cells in the secondary phloem, including the current sieve-tube layer (Fig. 1, B), were always full of starch grains. Starch was abundant in the epithelial cells surrounding the resin ducts

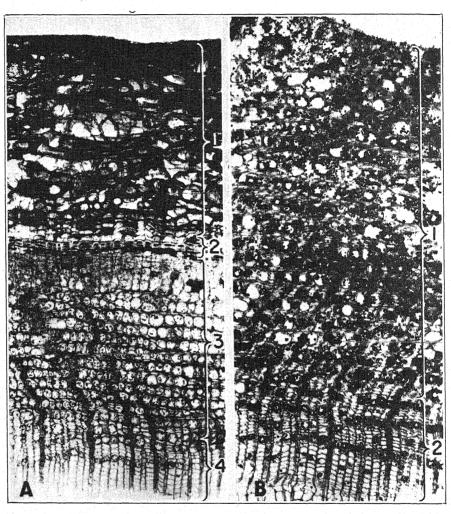


Fig. 1. Photomicrographs of shortleaf pine root bark. A. Transverse section showing (1) brown patch, (2) periderm layer, (3) secondary phloem, and (4) current secondary phloem layer. B. Transverse section showing accumulation of starch grains in (1) old secondary phloem and in (2) current secondary phloem layer. Starch grains stained black with iodine.

and in the medullary rays. Only a trace of starch was observed in the tissues of the trunk bark. Root bark is thicker than trunk bark and has larger and more abundant parenchyma cells for storing starch.

In both the root and trunk bark, the parenchyma cells (Fig. 1, A and B) used for storage arise in tangential rows in the sieve-tube layer and then

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increase in size as they are pushed outward by new growth, forming closely compacted rows between narrow bands of crushed sieve-tube layers. The secondary phloem of pine roots is an important reservoir for the storage of reserve food. Though little information is available on the use of the starch stored in pine roots, it is suspected that a large part of it is used for the growth and development of the root system. Regardless of the ultimate use of the root foods, it appears that the food storage capacity would be decreased if excessive brown patch developed at the expense of the storage tissue beneath.

A survey (Table 2) showed that the roots of diseased shortleaf pines had nearly 6 times as much brown patch as the healthy shortleaf pines within the little-leaf range, and 32 times as much brown patch as the healthy shortleaf pines growing outside the range. Brown patches were found on roots of healthy loblolly and longleaf pines within the little-leaf range, and on Virginia pine outside the range, but the percentage of root bark affected

	occurrence					

Localitya	Species	Condition of trees	Trees	Roots	Roots with brown patch	Average diam.	Area with brown patch	Age of trees
	4.5		No.	No.	No.	Inches	Per cent	Years
S. C., Ala.*	Shortleaf	Little leaf	22	43	43	1.8	96	31
S. C., Ala.*	Shortleaf	Healthy	23	45	31	1.5	17	29
Ga., Miss	Shortleaf	Healthy	39	78	32	1.6	3	47
S. Ć., Ala.*	Loblolly	Healthy	10	20	5	1.7	7	29
Miss	Loblolly	Healthy	9	18	3	1.5	1	34
Ala.*	Longleaf	Healthy	9	17	5	1.6	14	36
Ga	Virginia	Healthy	5	10	5	1.5	4	45

^a Asterisk indicates localities within the known range of little-leaf disease. All others were outside the range.

was small, being 14 per cent or lower. The amount of brown patch was somewhat larger on healthy loblolly and shortleaf pines growing in little-leaf localities than on the same species growing in healthy stands outside the little-leaf range. It is the abundance rather than the presence of brown patch on the roots of pines that must be regarded as an important symptom of the little-leaf disease.

Brown patch is regarded as the excessive and premature formation of rough bark. The cause of the larger amount of brown patch on the roots of little-leaf trees is not definitely known. Though little information is available on the histology of pine bark, it is possible that the initiation of brown patch results from the devitalization of the older part of the phloem layer. Such devitalization may result from aging, the effects of pathogenic organisms, a deficiency of essential elements, or the accumulation of toxic substances.

Pitch Cankers on Roots

Large, pitchy, cankerlike lesions have been observed frequently on the primary and lateral roots of healthy and diseased shortleaf pines, but

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mostly on diseased trees. Siggers and Doak (11) also reported that they found roots with black masses of pitch and soil adhering to the bark, and with the underlying woody part of the root heavily infiltrated with resin. Pitch cankers are from a few inches to about a foot long, depending on the

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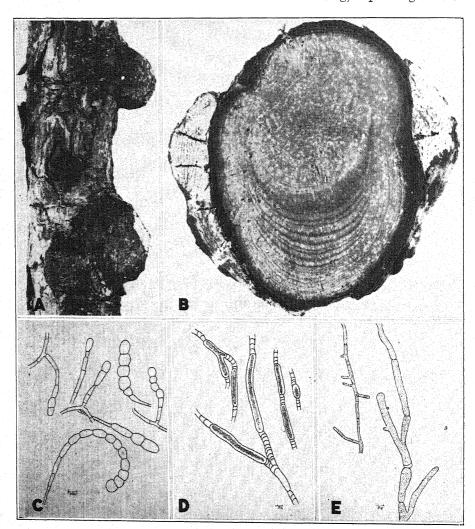


Fig. 2. Photographs showing characteristics of fungi associated with shortleaf pine roots. A. External appearance of tuckahoes of *Poria cocos*. B. Transverse section showing internal structure of tuckahoes of *Poria cocos*. C. Conidia and conidiophores of *Torula marginata*. D. Chlamydospores and heterocyst-like cells of *Poria cocos*. E. Small and large hyphae of *Poria cocos*.

size of the root. The bark is deeply cracked and the underlying wood is infiltrated with pitch, the extent of infiltration varying from a few rings to the entire stele in extreme cases. These lesions are usually covered with large masses of pitch-infiltrated soil firmly cemented to the bark. The soil masses are covered with dark hyphae resembling those of the *Torula* found in the

brown patches and giving them a dark sooty appearance. Detailed studies of root defects have not progressed far enough to offer any conclusions regarding the cause and significance of the pitch cankers.

FUNGI

Occurrence of Tuckahoes on Roots of Diseased and Healthy Shortleaf Pines

Root excavations showed that tuckahoes occur frequently on roots of diseased shortleaf pines but they have not been found on the roots of healthy trees. Tuckahoe is a common name of the sclerotium of a soil-inhabiting fungus known as *Poria cocos* Wolf. Isolations from the tuckahoes on shortleaf pines yielded a fungus that has been identified as a strain of *Poria cocos*. The tuckahoe fungus has not been isolated directly from such root defects as pitch cankers and dieback of the feeding roots.

The tuckahoes (Fig. 2, A) on roots of diseased pines vary from one-half to two inches and appear as irregular lumps on the surface of the bark. A fresh sclerotium (Fig. 2, B) is a compact, white mass of fungus cells with a cheesy consistency, striated with thin layers of bark, which becomes hard and fissured when dry. The tuckahoes (Fig. 2, A) on the roots of little-leaf trees differed markedly, both in size and shape, from the large tuberous tuckahoes that have been described previously (13, 15, 16).

Fungi Associated with Brown Patch

A species of *Torula* was consistently isolated from brown patches on roots of diseased shortleaf pines as well as roots of healthly shortleaf, loblolly, longleaf, and Virginia pines in the little-leaf belt. The *Torula* was isolated also from recently formed brown patches on healthy shortleaf, loblolly, longleaf, and Virginia pines growing outside the present known range of the little-leaf disease. This brown patch *Torula* is apparently widely distributed on the roots of pines in the Piedmont.

In 1939, Siggers⁴ isolated a fungus from roots of little-leaf and healthy pines in Alabama, and from diseased roots of slash pine (*Pinus caribaea* Morelet) seedlings in a forest-tree nursery in Mississippi. This fungus has the same cultural characteristics as the brown-patch *Torula*.

Cultural Characteristics of the Brown-Patch Torula

The brown-patch Torula is more closely related to Torula ligniperda (Willk.) Sacc. (9, pp. 565-566; and 10) than to any of the other species of Torula described by Saccardo (9). The brown-patch fungus differs from T. ligniperda, as shown by comparisons with an authentic culture and a description by Siggers (10) of T. ligniperda, in having: (1) larger conidia, (2) longer chains of conidia, (3) a sharply defined white margin of the mycelium, and (4) a more compact and faster-growing mat of mycelium.

⁴ P. V. Siggers, Associate Pathologist, U. S. Dept. of Agr., Bureau of Plant Industry, Soils and Agricultural Engineering, Division of Forest Pathology, Saucier, Mississippi.

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On the basis of these differences, the brown-patch fungus is described as a new species of *Torula*. The characteristics of the conidia are shown in figure 2, C.

Torula marginata sp. nov.

Mycelium (on potato-dextrose agar) black with white margin, floccose in central part, appressed at outer part, growth very slow, with numerous black flecks in agar; mats fractured radially; hyphae 2–4 μ wide, dark, septate, without clamps, occasionally anastomose, walls sometimes roughened; conidia catenate, 2–12 or more but mostly 6 in a chain, oblong to (becoming) spherical, thick-walled, 1-guttulate, dark-brown, smooth, 12–13 ×11–12 μ , on short simple conidiophores, often appearing intercalary by the germination of the terminal conidium.

Habitat. Associated with dead bark on roots of *Pinus echinata*, *P. tacda*, *P. palustris*, and *P. virginiana* in the southeastern United States. Type specimen, Forest Pathology No. 90914, deposited in Mycological Collections, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

Coloniae⁵ in agaro solani-dextroso atrae margine distincto albo, centro floccosae, margine appressae, tarde crescentes, radiatim fringentes, ex hyphis 2–4 μ latis fuscis septatis non fibulatis interdum anastomosantibus saepe verrucosis compositae; puneta numerosa nigra in agaro dispersa; conidia in catenulis cellularum 2–12 vel plurium plerumque 6 disposita, oblonga usque spherica, ex extremo proximo ad distantem gradatim magnitudine crescentia, crasse tunicata, 1-guttulata, atro-fusca, levia, 12–13 × 11–12 μ , in conidiophoris brevibus simplicibus orta; conidia terminalia catenularum submersarum germinantia et hyphas producentia ut catenulae intercalares videantur.

Hab. cum cortice emortuo consociata in radicibus Pini echinatae, P. taedae, P. palustris, et P. virginianae, in regione austro-orientali, U. S. A.

Cultural Characteristics of the Tuckahoe Fungus

The fungus isolated from tuckahoes on pine roots is regarded as a strain of Poria cocos. Its growth on potato-dextrose or 2 per cent malt agar was rapid and uniform, the fungus forming 90-mm. mats in 3 days at 26° C. The mycelium is cottony and white in 10-day-old cultures but becomes appressed and frequently is tinged with some shade of brown with age. The mats are soft, friable, and odorless, and they do not discolor the media. All of the cultures were white when first isolated. The mycelium is fragrant when grown on large quantities of cooked wheat. The fungus usually produces lacerate-poroid, white, resupinate sporophores on 10-day-old mats in plates and tubes. These sporophores produce viable basidiospores. The mycelium is composed of small and large hyphae (Fig. 2, E). The small hyphae are $2-4\mu$ in diameter, hyaline, thin-walled, septate, and without clamps. The large hyphae are $7-12 \mu$ in diameter, hyaline when first formed, but turn to pale yellow with age, septations slightly constricted, and without clamps. In old cultures, the large hyphae (Fig. 2, D) are transformed into long, thick-walled, pale yellow chlamydospore-like cells that are separated by rows of narrow, empty heterocyst-like cells. The length of the chlamydospore-like cells is variable but the average size of 10 cells from each of 3 different isolates was $52-71 \times 7-12 \mu$.

An occasional culture developed a dark-brown mat. The dark-brown cultures always reverted to the typical white form when replanted on agar.

⁵ The Latin description was prepared by Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

The mycelium is appressed and white at first, soon turning to cinnamonrufous or chestnut-brown and becoming fragrant. The medium changes to dark brown. The dark-brown form of the mycelium can be produced by crossing different isolates but not by crossing different cultures of the same isolate. A zone of dark-brown mycelium forms across the plate along the juncture of the colonies.

The morphological features of the *Poria* isolated from tuckahoes on roots of little-leaf trees are comparable with those given for *Poria cocos* by Davidson, Campbell, and Vaughn (5), Wolf (15), and Weber (13). Variations in the color of the mycelium of the little-leaf isolates and the isolates reported by other investigators (5, 13, 15) are regarded as strain differences. The formation of chlamydospore-like cells, fragrance of mycelium on cooked wheat, and the dark-brown form of growth are new features for *Poria cocos*.

INOCULATIONS

Pathogenicity of Torula marginata and Poria cocos on Roots of Adult Healthy Shortleaf Pines

Primary roots of shortleaf pines 4 to 6 inches in diameter at breast height were inoculated, in situ, with *Torula marginata* and *Poria cocos* to determine whether or not these fungi would induce the development of brown patch. The trees were in healthy stands of pine at Dadeville, Alabama, and Calhoun Falls, South Carolina. Each root was given two inoculations spaced 24 to 30 inches apart, one with fungus inoculum and the other with sterile cooked wheat. The root bark was wounded by cutting 6 slits about one-fourth inch long. The inoculated roots were removed for examination during the sixth and eighth months after inoculation. The amount of brown patch was calculated as the percentage of total bark surface affected within the limits of its maximum linear extent.

In both localities, the inoculations (Table 3) with Torula marginata resulted in brown patch, and the number of affected roots varied from 67 to 100 per cent per series. All of the isolates, except Torula 69, produced lesions with deep resinous cracks, sometimes extending to the wood, large pitch pockets in the bark, and pitch infiltration in the underlying wood as far as the third ring. Large masses of pitch-infiltrated soil were attached to some of the lesions associated with deep wood infections and they had the characteristics of pitch cankers. The number of lesions with pitch pockets and pitch-infiltrated wood in the series with Torula 50 and 56 was larger in South Carolina than in Alabama. Although the reason for this difference is not known, it is suspected that it may be a difference in soil conditions. Torula marginata was reisolated from brown patch on inoculated roots in both localities.

There was no evidence of brown-patch formation or infection where the roots had been inoculated with sterile wheat and in most instances the bark incisions had completely healed.

⁶ Colors according to Ridgway (8).

The isolates of *Poria cocos* caused brown patch on roots in both localities (Table 3), and the percentage of affected roots varied from 50 to 100 per series. In addition to brown patch, the isolates produced deep lesions that sometimes extended into the wood, pitch pockets, and heavy exudation of pitch. Lesions resulting in deep wood infections were covered with large masses of pitch-infiltrated soil and had the characteristics of pitch cankers. There was no evidence of brown patch or infection where the roots had been inoculated with sterile wheat and most of the bark incisions had healed. *Poria cocos* was reisolated from brown patches on inoculated roots in South Carolina and Alabama.

TABLE 3.—Pathogenicity of Torula marginata and Poria cocos on roots of healthy shortleaf pines 4 to 6 inches in diameter at breast height

	Aver- age	Inocu-	Inocu- lated roots	Bark surface affected	Brow	n patches	with	Inocu- lated roots
Fungi	root diam- eter	lated roots ²	with brown patches ^b	by brown patches	Cracks	Pitch pockets	Resi- nosis ^d	with wood infec- tion
	Inches	Number	Percent	Percent	Percent	Percent	Percent	Percent
Torula 50e	0.7	16	88	69	57	0	100	21
" 50*f	0.9	5	100	60	60	40	100	100
'' 56	0.6	16	94	81	60	20	93	27
· · 56*	0.8	14	100	64	36	50	100	57
" 69	0.6	3	67	45	50	0	0	0
Poria 12	0.7	15	100	82	93	20	93	67
" 12*	0.8	8	88	52	43	43	100	29
" 15	0.8	4	50	23	100	0	100	50
· · 53	0.7	14	100	79	79	7	86	14
" 53*	0.8	9	67	59	33	33	100	17

^a Each root received 2 inoculations, one with fungus and the other with sterile wheat.

^b None of the inoculations with sterile wheat produced brown patch or infections; therefore, data were omitted in table.

d The term "resinosis" means heavy external flow of pitch.

e Stock culture number of the isolate.

Torula marginata and Poria cocos thus are capable of not only producing brown patches but also of causing infections that extend into the wood on roots of healthy shortleaf pines growing under natural conditions.

Pathogenicity of Torula marginata and Poria cocos on Roots of Shortleaf Pine Seedlings

The possibility that the dieback of the feeding roots on little-leaf trees is caused by fungi was studied further by testing the pathogenicity of *Torula marginata* and *Poria cocos* to the roots of 2-year-old shortleaf pine seedlings. Nonsterile soil was used in this experiment. The roots of the seedlings were inoculated at the time of transplanting by scattering the grains of cooked wheat inoculum through the soil about one-half inch from the roots. The controls were inoculated with sterile cooked wheat.

c Calculated as per cent of bark surface within maximum linear extent of brown patch.

f Inoculations marked with an asterisk were made in South Carolina; the others were made in Alabama.

All of the isolates of *Poria cocos* spread rapidly in the soil and caused heavy losses (Table 4) of plants in a relatively short time. For all isolates, the number of plants killed in 12 to 90 days varied from 79 to 100 per cent per series. In single series with isolates 53, 15, and 12, from 88 to 100 per cent of the plants were dead on the twelfth day after inoculation. The mycelium spread rapidly through the nonsterile soil and poroid mats formed on the surface of the soil in 14 days in some of the pots.

The feeding roots of the affected plants were decayed and the infections in the taproot extended upward for varying distances, and in a few instances involved the entire length. The bark on infected taproots was loose and dark brown and the stele was light brown, both being sharply demarcated

TABLE 4.—Pathogenicity of Torula marginata and Poria cocos on roots of 2-year-old shortleaf pines grown in pots of unsterilized soil

Tot	al	D1		701 1 7 7	
Pots	Plants	Duration	Inoculum	Plants dead	
No.	No.	Days		Per cent	
6	24	185	Torula 50a	79	
6	24	185	Torula S4b	83	
4	16	185	Torula 56	75	
6	24	185	Sterile wheat	21	
6	24	185	Sterile wheat	13	
6	24	12	Poria 53	100	
6	24	90	Poria 53	79	
6	24	12	Poria 15	100	
6	24	10	Poria 12	88	
4	16	90	Poria 12	100	
6	24	78	Sterile wheat	8	
6	24	90	Sterile wheat	0	

^a Stock culture number of the isolate.

from the live tissues. *Poria cocos* was reisolated from the steles of the infected taproots. Roots of all sizes killed by *Poria* were soon invaded by *Trichoderma*, which caused the steles to turn dark green or black.

The isolates of *Torula* (Table 4) were definitely slower in their action than those of *Poria*, and the seedling losses occurred sporadically throughout a much longer time. The seedling losses in single series of 3 different isolates were 75 to 83 per cent during 185 days. The losses in the two control series were only 13 and 21 per cent. *Torula* S4, which is one of the isolates from a Mississippi nursery, caused a slightly higher loss than the brownpatch isolates. Although the losses from *Torula* are high, they are not quite so high and did not occur so rapidly as the losses from *Poria*.

Both *Poria cocos* and *Torula marginata* were pathogenic to the roots of 2-year-old shortleaf pine seedlings.

The feeding roots of the affected plants were decayed and the deeper parts of the taproots were killed. By the end of the experiment the survivors among seedlings inoculated with *Torula* had produced much less shoot

^b P. V. Siggers isolated *Torula* S4 from roots of slash pine seedlings at a forest nursery in Mississippi.

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de of growth than the plants in the controls, which indicated that the *Torula* may have a stunting effect on shoot growth.

A further test of the pathogenicity of *Poria cocos* and *Torula marginata* was made by inoculating the roots of 2-year-old shortleaf pines that had been potted for several months. The roots were inoculated by placing a table-spoonful of cooked wheat inoculum in the soil about one inch from each seedling. The controls were inoculated with sterile wheat.

The plants in all of the series remained healthy but dormant from the time of inoculation in November, 1942, until the last week in April, 1943. Dying of seedlings started during the last week in April, 1943, and it continued throughout May. The percentage of dead plants was high for all of the series inoculated with fungi, averaging 70 per cent for the 2 series with *Poria*, and 58 per cent for the 2 series with *Torula* (Table 5). Seedling

TABLE 5.—Pathogenicity of Poria cocos and Torula marginata on the roots of established shortleaf pine seedlings in pots

Inoculum	Pots	Plants inoculated	Dead plants
Sterile wheat	No. 25 14 8 10 16	No. 70 45 20 32 45	Per cent 9 71 70 63 53

a Stock culture number of the isolate.

losses in the control series were low, averaging only 9 per cent. Both *Poria cocos* and *Torula marginata* are pathogenic to the roots of established shortleaf pine seedlings.

The roots of 2-year-old shortleaf pines were inoculated with *Poria cocos* according to Wellman's (14) technique. The fungus was grown in a liquid medium consisting of 0.004 M KH₂PO₄, 0.008 M Ca(NO₃)₂, 0.004 M MgSO₄, and filtered extract of 200 g. of potatoes per liter. The inoculated seedlings were planted in pots of nonsterile soil. An attempt was made to determine whether or not the addition of humus to the soil would affect the pathogenicity of the fungus by potting the seedlings in one of the three series in a mixture of 2 parts of coarsely screened woods humus and 3 parts of soil, by volume. Each series consisted of 6 pots with 4 seedlings per pot.

The seedlings in each series remained healthy but dormant from the time of inoculation in November, 1942, until the last week of April, 1943. Dying of seedlings in all of the series started during the last week of April at the time of new shoot growth and continued throughout May. The total seedling losses were high in all of the series: 54 per cent for *Poria* 53 in soil; 75 per cent for *Poria* 53 in soil with added humus; 79 per cent for *Poria* 15 in soil. The addition of humus to the soil at the time of inoculation did not have an appreciable retarding effect on the pathogenicity of the fungus.

Further work was started during the summer of 1943 to test the effect of the addition of woods humus to the soil on the pathogenicity of *Poria cocos*

and $Torula\ marginata$ to the roots of pine seedlings. Two parts of coarsely screened woods litter were mixed with 3 parts of sandy loam soil, by volume. Three-year-old shortleaf pines were planted at the time of inoculation in metal flats measuring $12 \times 24 \times 8$ inches. The roots were inoculated by mixing 2 tablespoonfuls of granular cooked wheat inoculum with the soil used for setting the roots of each seedling.

The percentages of dead plants (Table 6) resulting from inoculations with *Poria cocos* and *Torula marginata* were large in both the soil and the soil plus humus series, varying from 53 to 83 per cent per series. The percentages of dead plants in the control series were low, being 5 per cent or less for both soils. Since the losses from *Poria* were larger in the straight soil series, and the losses from *Torula* were larger in the soil plus humus series, it is concluded that the addition of humus at the time of inoculation did not definitely affect the pathogenicity of these 2 fungi.

TABLE 6.—Effect of addition of humus to soil on pathogenicity of Poria cocos and Torula marginata to roots of shortleaf pine seedlings

Kind of soil	Inoculum	Flats	Plants inoculated	Dead plants
		No.	No.	Per cent
Straight soildo do do Soil plus humusdo do do do do do	Sterile wheat Torula 56a Poria 53 Sterile wheat Torula 56 Poria 53	6 3 3 6 3 3	60 30 30 60 30 30	3 53 83 5 60 63

a Stock culture number of the isolate.

The results of the seedling inoculations have demonstrated that Poria cocos and Torula marginata are capable of destroying the fine lateral roots as well as the taproots of shortleaf pine seedlings. *Poria cocos* was definitely more virulent, as shown by the higher percentages of dead plants, than Torula marginata in all of the tests. The pathogenicity of the tuckahoe fungus (Poria cocos) confirms Wolf's (15) assumption, which was based on a detailed study of the characteristics of the tuckahoes of Poria cocos on pine roots, that the fungus is probably parasitic. In a later paper, Wolf (16) reported that the tuckahoe fungus on maize was a pure saprophyte, but he reiterated the fact that on pine the evidence indicates that the sclerotia are the result of pure parasitism. Weber (13) did not state whether or not the tuckahoe fungus (Poria cocos) was parasitic on the roots of orange trees. More recently, Davidson, Campbell, and Vaughn (5) reported that Poria cocos causes a brown rot of roots and trunk heartwood of oak and other hosts. The results of the present investigation indicated that the tuckahoe fungus must be regarded as a root pathogen. Although it has been demonstrated that the strain of Poria cocos isolated from tuckahoes associated with roots of little-leaf trees is pathogenic to the roots of pine seedlings, it is not known whether the fungus is capable of attacking in like manner the feeding roots of adult pines.

A review of the literature failed to reveal any information on the pathogenicity of species of *Torula* to the roots of pine. According to Boyce (1), *Torula ligniperda* is associated with discolorations in hardwoods, and the fungus has been found causing stain in the heartwood of living paper birch and yellow birch. Cooke (4) has reported that Ravenel found *Torula diversa* Cke. on leaves of *Agave* at Darien, Georgia. The results of the present investigation have demonstrated that the *Torula* associated with brown patches is pathogenic to the roots of pine seedlings. As in the case of *Poria*, it is not known whether the fungus is also capable of attacking the feeding roots of adult pines.

SUMMARY

A serious deterioration and dying of southern pines known as little leaf is widely distributed in the Piedmont region of several Southern States and the upper coastal plain of Alabama. Little-leaf trees have yellowed needles, shortened internodes of the twigs, and reduced diameter growth, and they die prematurely. This paper has presented the results of studies on the destruction of roots by pathogenic fungi.

No pathogenic fungi that appear to be responsible for the little-leaf disease were found associated with the aboveground parts.

The root systems of little-leaf trees are consistently more defective than those of healthy trees. The root defects that have attracted attention are dieback of feeding roots, dead primary and lateral roots, pitch cankers on the larger roots, and the excessive exfoliation of bark on the smaller roots, known as brown patch.

There was no observable difference in the gross morphology of the root systems of diseased and healthy trees. Roots of all sizes on little-leaf trees and large primary roots of healthy trees are usually covered with brown patch. On healthy trees, the roots less than one inch in diameter have only small and widely scattered brown patches.

There was no observable difference in the condition of the mycorrhizae of little-leaf and healthy trees.

Brown patches occur normally on roots of healthy trees of 4 species of southern pines. It is the abundance rather than the presence of brown patches on pine roots that is regarded as an important symptom of little leaf. A species of *Torula* is consistently associated with newly formed brown patches.

Root-bark phloem contains abundant starch over much of the year. An abundance of deep brown patches, such as occurs on roots of little-leaf trees, must result in a reduction of the food-storage capacity of the roots.

Pitch cankers occur on the roots of diseased and healthy shortleaf pines. Sclerotia of a soil-inhabiting fungus known as *Poria cocos* were found frequently on roots of little-leaf trees but not on healthy trees.

The cultural characteristics of the strain of *Poria cocos* isolated from tuckahoes and the species of *Torula* associated with brown patches are described. The new specific name of *Torula marginata* has been given to the brown patch *Torula*.

Poria cocos and Torula marginata produced brown patches and wood infections when inoculated on roots of adult shortleaf pines. When inoculated on the roots of shortleaf pines 2 and 3 years old grown in pots of nonsterile soil, both fungi attacked the roots and killed a large percentage of the plants. The percentages of dead plants were higher in the Poria series than in the Torula series. The addition of woods humus to the soil at the time of inoculation failed to affect the pathogenicity of Poria and Torula.

DIVISION OF FOREST PATHOLOGY: BUREAU OF PLANT INDUSTRY, Soils, and Agricultural Engineering; Agricultural RESEARCH ADMINISTRATION; U. S. DEPARTMENT OF AGRICULTURE, in cooperation with

GEORGE FOSTER PEABODY SCHOOL OF FORESTRY, University of Georgia, Athens, Georgia.

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RESERVE FOOD STORAGE IN SHORTLEAF PINE IN RELATION TO LITTLE-LEAF DISEASE

GEORGE H. HEPTING

(Accepted for publication September 20, 1944)

INTRODUCTION

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The symptoms of the little-leaf disease of pine are strongly suggestive of malnutrition. Twig growth is abnormally short, the needles are short and usually yellowish, the radial growth of the trunk is greatly reduced in the later stages, and by the time the trees die the crowns are very thin. The extent to which the various organs decline as the disease progresses has been presented in detail by Buchanan (2). In studying the roots of little-leaf trees Jackson (9) found that the scaly, scabby root condition called brown patch is due to excessive and premature formation of rough bark. eased trees the cork cambium cuts deeply into the phloem over wide areas of small roots, resulting in the loss of much of this tissue, wherein the greater portion of the root's food reserves is stored. Since at least one and possibly two fungi appear to be associated with the brown patches, it seemed possible that little leaf was the result of root starvation owing to reduction of the food storage capacity of the roots by excessive brown patch (9). other hand, excessive brown patch may follow root starvation, as a result of reduced synthesis of food in the crown due to some undetermined cause. A knowledge of food storage conditions, particularly in the root, seemed necessary to an understanding of the reasons for the symptoms and behavior of the disease.

A generally low carbohydrate content throughout a little-leaf tree would indicate a systemic disturbance such as might be induced by a virus disease or unfavorable soil conditions, or possibly by the action of organisms detrimental to the absorptive system. Localization of deficient or excessive reserve foods might indicate specific disorder of certain organs. For example, White-Stevens (18) points out that several investigators have demonstrated that the foliage of boron-deficient plants has an excessive accumulation of carbohydrates whereas the roots of such plants are strikingly deficient in food reserves.

The present study involved the quantitative analysis of reserve foods in the shortleaf pine (*Pinus echinata Mill.*), from samples taken each month of the year (except one) from healthy trees in Alabama, within an area where

¹ Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture, in cooperation with the Appalachian Forest Experiment Station, U. S. Forest Service, Asheville, N. C., and Alabama Polytechnic Institute.

The author gratefully acknowledges the field and laboratory assistance of Elmer R. Roth, E. Richard Toole, David W. Morison, and W. R. Boggess. The Champion Paper and Fibre Co., through Chief Chemist Fred Doutt, permitted the use of their grinding equipment, and was of considerable help in working out the methods and interpretations of the analyses.

little leaf is common, from diseased trees in this same area, and from healthy trees near Asheville, N. C., about 40 miles from the nearest known little-leaf disease. Since the roots of diseased trees are altered in a way that might explain the trees' decline, attention has been focused on the reserve food content of the roots, supplemented with parallel data on the trunks, throughout the course of one year, and data on food in the needles at one period in the spring.

SOME EARLIER WORK ON DISTRIBUTION OF CARBOHYDRATE RESERVES IN TREES

Food reserves in plants are of many types, consisting mainly of carbohydrates, proteins, fats, and oils. In trees the carbohydrates, largely starch, are the most prevalent. Sinnott (16) and Preston and Phillips (11) have reviewed early work dealing with the possibilities that hemicellulose² is also a functional food source. The role of hemicelluloses as reserve foods has not been convincingly demonstrated, and opinion prevails that if they have such a role, it may be only under extreme circumstances when starch and sugars have been exhausted. Very little of the earlier work on reserve foods was done on roots, and most of the observations were based upon qualitative iodine tests for starch, rather than upon chemical analyses.

Preston and Phillips (11) have given an excellent review of the state of knowledge of tree reserve foods in 1911. Their review and experiments indicated the following:

1. During the winter the starch content drops to a minimum in the stems and twigs of most trees in temperate climates.

2. A few species, both hardwood and softwood, show considerable increase of fat in phloem and xylem in winter, but not enough to account for the amount of starch that disappears.

3. In the roots the transformations do not keep pace with those in the stem, and starch remains the entire year, with the greatest reduction occurring in spring.

4. Fabricius (4) stated that the older stem of *Picea excelsa* does not transform its starch to so great an extent as do the younger stems. Preston and Phillips' own work also showed less change in older stems.

5. Sablon (13) maintained that the maximum for total carbohydrate reserves for deciduous trees is at the time of leaf fall in the autumn, whereas the maximum for evergreen trees is at the time of opening of buds in the spring.

6. Sablon (12) and Schellenberg (14) concluded that a principal carbohydrate reserve of trees in winter is hemicellulose, and attempts were made by the Europeans to demonstrate this both by chemical analysis and by evidence of changes in the thickness of cell walls and wall erosion, in the spring.

Sinnott (16), following the work of Fischer (5), classified a large number

² The term is here used as a collective one, for those polysaccharides that bridge the gap between true cellulose and the clearly recognized reserve carbohydrates like starch (7, p. 40).

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of genera of trees and shrubs as "starch trees" or "fat trees," depending upon the predominant food reserve in pith and wood of twigs and young branches during mid-winter. Most of the gymnosperms were classified as fat trees, a few as starch and fat trees, and none were called starch trees, while the order was reversed for angiosperms. Since Sinnott's test for fat was simply staining with Sudan III, he must have classed as reserve fats the resins of the conifers he worked with. In fact, he mentions their accumulation in ray parenchyma and resin canal epithelium. These resins stain readily with Sudan III but generally are not regarded as food reserves, being the oxidation products of terpenes, and largely irreversible. Miller (10) states that aside from their protective influences, no biological use of these substances is known. The interpretation of Sinnott (16), Fischer (5), and others, to the effect that these resins are food reserves affects acceptance of their views on food transformations. Sablon (12) did not regard the fats as major food reserves, but maintained that when starch decreased in winter the conversion was largely to hemicellulose, and that digestion of this reserve hemicellulose was an important source of carbohydrate in the spring.

Sinnott (16) states with respect to stems, twigs, and small branches, that "in all species starch disappears in the fall almost completely from phloem and cortex, and even in the starch trees it is much reduced in the wood as well." The present work refutes this for shortleaf pine. He further states that his data show that the character of the food reserve in any cell depends primarily upon the ease with which water or substances carried by water have access to the cell. Where the movement of liquids is slow and difficult, the reserve persists as starch; where such movement is easy, starch disappears at the beginning of winter and fat is produced. He does not say whether he considers that this fat changes back to a carbohydrate again to be used by the plant in the spring. Since much of his "fat" was resin, such change apparently cannot take place.

Traub (17) found that the reserve carbohydrates in peach twigs reached their maximum just after leaf fall, and that they were made up largely of pentosans and to a much less extent of hexosans.

In reviewing past work it is necessary to keep in mind continuously the organ discussed, whether young twig, old trunk, or root, and the kind of plant—deciduous or evergreen, tall tree or small shrub. Too often authors refer to the carbohydrate content of a tree at a certain time when they really mean a specific organ such as the twig, or the root. Few workers have studied reserve food changes in all important organs of a plant simultaneously.

METHODS

From the high starch content in the outer phloem of shortleaf pine roots over much of the year, easily demonstrable with or without the use of iodine-potassium iodide (IKI) (Fig. 1, B, by Jackson (9)), it was obvious that starch is undoubtedly a very important food reserve in the root. In quantitatively studying these reserves from the standpoint of comparing little-leaf

trees with healthy trees, analysis must include at least starch, the sugars, and the carbohydrates intermediate between them. Since the question of whether hemicelluloses normally function as reserve foods is not settled, the analysis did not include hemicelluloses. Neither was an analysis of pentosans made. From the fact that in the shortleaf pine the storage tissues of wood and bark are gorged with starch over much of the year, it appeared most reasonable to use as an index of general organic nutrition the sum of starch, sugars, and their intermediate products. To determine the best method of hydrolyzing the carbohydrates to glucose, samples from a single lot of ground root bark were digested with takadiastase, digested with a proprietary diastase preparation, or were autoclaved with 3 per cent hydrochloric acid, which not only hydrolyzed the starch but the hemicellulose as The effect of clearing with lead acetate following hydrolysis was also tested. Table 1 gives the results of these preliminary tests, using the Shaffer-Somogyi method of sugar analysis, as described by Heinze and Murneek (8).

TABLE 1.—Amount of reserve carbohydrate in root bark as determined by enzyme and acid hydrolysis

${f Treatment}^a$	Reserve food, in terms of glucose, as a percentage of oven-dry weight of bark
Takadiastase, not cleared Proprietary enzyme compound, not cleared Proprietary enzyme compound, cleared Acid hydrolysis, not cleared Acid hydrolysis, cleared	19.5 14.2 11.3 31.2 17.0

a Eight replications of each test.

Takadiastase seemed to hydrolyze the reserve carbohydrate somewhat more completely than the other enzyme preparation, and was far less drastic than the acid hydrolysis, which took out considerable substance that might not serve as reserve food. It is felt that the hot-water-soluble carbohydrates produced by takadiastase digestion plus the other water-soluble carbohydrates come close to representing the more important food reserves of the shortleaf pine. By adopting this method for all subsequent analyses, all data are comparable and the nature of the carbohydrates determined is understood.

Each month two little-leaf trees and two nearby healthy trees were cut near Dadeville, Alabama, and two healthy trees near Asheville, North Carolina. The trees were between 6 and 8 inches in diameter, at breast height, and dominant or codominant. A 6-inch disc was cut from the trunk at 5 feet above the ground, and a 2-foot-long section from each of 2 roots about an inch in diameter was grubbed out. Preliminary tests had shown that the carbohydrate content of the trunk at 5 feet well represented the stem up to a height of 25 feet, that the bulk of the extending roots range from $\frac{1}{2}$ to $1\frac{1}{2}$

b Neutral lead acetate followed by potassium oxalate.

inches in diameter, and that within these limits root size had little effect on carbohydrate content on a dry-weight basis. These tests determined the type of sampling. The dimensions of the pieces were measured and the bark area and wood volume computed. All of the root wood and the bark from the two 2-foot root sections and the stem piece were ground in a Wiley mill. Since the stem block was larger than needed for a wood sample, two sectors of wood were cut out of each disc and their volumes computed. Immediately after collecting the samples in the field they were dried for two or three days at 75° C. to fix them. They were then ground. The sawdust was dried to constant weight at 75° C. Two 2-gram samples were taken from each batch. We thus had duplicate samples each month from two localities: two diseased and four healthy trees in all, one stem sample and two root samples from each tree, and from each sample both bark and wood tissue, separately.

The 2-gram samples were steeped in ether for three days to remove the resins; they were filtered and the residue and the filter paper returned to the flask; 100 ml. of distilled water were added to each and they were simmered for 20 minutes to saturate the sawdust; after cooling, 50 mg. of takadiastase were added to each and the flasks kept at 38° C. for 24 hours with frequent agitation; and then they were autoclaved at 15 lb. pressure for one hour, and filtered. Ten ml. of filtrate were drawn off if bark, and 25 ml. if wood, and this made up to 50 ml. with distilled water, and 5 ml. of concentrated hydrochloric acid were added. The flasks were then simmered for 2 hours in a reflux condenser to completely hydrolyze to glucose all carbohydrates now in solution. The solutions were neutralized with sodium hydroxide solutions, first with a saturated solution, finishing with a solution 10 per cent of saturation; 3 ml. of a saturated solution of neutral lead acetate were added to clear the tannins, etc.; the solutions were filtered, each onto 9 grams of potassium oxalate to remove the lead, filtered again and made up to 150 ml. with distilled water. Heinze and Murneek's (8) description of the Shaffer-Somogyi method was followed to determine the amount of glucose in the final solution. It is of interest that, whereas Heinze and Murneek got complete reduction by their alcoholic extracts of plant tissues after 15 minutes, a 45-minute heating period was required to get complete reduction with the present aqueous extracts. In the case of wood extracts, clearing with lead acetate had no effect upon the final result, and hence was not done in most of the work.

The method is remarkably precise. Duplicate samples gave almost identical results, while the slightest difference in amounts in the tissues showed up clearly. The procedure is time-consuming, and in the present case one month's samples were completed just in time for the next, with an assistant devoting full time to the 72 samples analyzed each month.

The analyses provided figures on the total carbohydrates, from starch and the higher sugars to glucose, all converted to and expressed in terms of glucose. This fraction will be referred to as reserve food. By weighing the total dried wood and bark samples, and by getting the volume of the wood

samples and the area of the bark samples, it was possible to express the reserve food in the following ways: for both bark and wood as a percentage of the total oven-dry weight, in the case of bark in terms of milligrams of glucose per square inch of bark, and in the case of wood in terms of milligrams of glucose per cubic inch of wood. For bark, expressing reserve food as a percentage of the oven-dry weight has disadvantages. Shortleaf pine trunks vary considerably in the thickness of the outer bark. Since the food reserves are largely confined to the living cortical tissue, if one tree had thick outer bark, its food content, expressed in terms of percentage of the ovendry weight of the bark would be very much lower than another tree with thin outer bark, but the same amount of inner bark. For trees of 6 to 8 inches' diameter, the bark thickness often varied an inch or more between trees. To minimize the influence of the outer bark on the final figures, the stem sections were peeled close to the inner bark before stripping the bark for grinding and weighing. The root bark was not trimmed or scraped, but only the dirt was washed off, before peeling and grinding.

RESULTS

The great bulk of the bark starch is in the outer phloem. There is also abundant starch in the ray parenchyma, and in those phloem parenchyma cells, scattered among the sieve tubes, which will later expand to form the major phloem storage tissue. Starch is regularly absent from the sieve tubes and from the cortical cells cut off by the phellogen and constituting the outer bark. These patches of outer bark on small roots are referred to as brown patch, since they give the impression of being dead lesions on the normally reddish turgid bark of these roots. In the wood, starch occurs in the living elements such as ray parenchyma, pith, and resin canal epithelium.

The Annual Cycle of Reserve Foods in Trunk and Root

Figure 1 presents the carbohydrate cycle over the course of one year for healthy and little-leaf trees. This figure is put on a basis of milligrams of glucose (all starch and sugars being converted to glucose) per square inch of bark and per cubic inch of wood. These have proven to be better bases on which to follow the cycles than is the percentage of the oven-dry weight, since the curves are more regular. Table 2 presents the ranges covered by figure 1 in terms of percentage of the oven-dry weight.

The following conclusions may be drawn from figure 1:

- 1. There was good agreement between the cycles for healthy trees from North Carolina and Alabama. The sudden dip of the June point for Alabama root bark is unexplained. The North Carolina curves, based on trees far from the little-leaf belt, were more regular than the Alabama curves, based on trees intermixed with little-leaf trees.
- 2. Roots of little-leaf trees had much less reserve food than roots of healthy trees, and their graph shows no cyclical pattern because the great variation between diseased trees overshadows the cyclical changes.

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3. The stem and roots of normal trees had their maximum accumulation of reserve food in the early spring. This checks with Sablon's (13) observations on *Pinus australis* Michx. f. Minimum reserves occurred in the roots in the fall, and in the stem the minimum was maintained all year except for a short high period in the spring.

4. Little-leaf root bark samples taken in February, May, and July were almost completely depleted of food. Although tests showed between 60 and 75 milligrams of glucose per square inch (about 8 per cent), inert outer bark will test over 4 per cent because of substances that, although not associated with living tissue, are converted to glucose in the process of extraction.

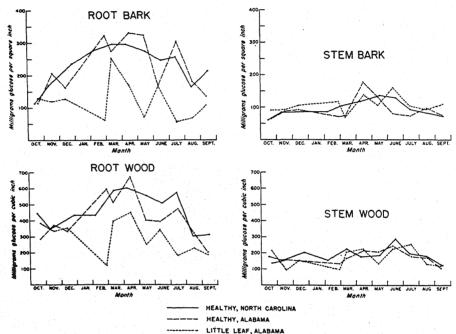


Fig. 1. Annual cycle of reserve food changes in shortleaf pine stems and roots.

Duplicate runs from the same batch of sawdust gave almost identical results. Variation in carbohydrate content between healthy trees or between roots of the same healthy tree, for a given month, was usually not great. The variability between roots of the same tree, expressed as a percentage of the mean for the two roots of each tree at each month, averaged 26 per cent, and when expressed as an average did not differ between diseased and healthy trees. There was more pronounced variability, however, between the roots of certain diseased trees. In October, healthy roots were near food exhaustion, but certain little-leaf roots were extremely low at times of the year when normal roots were at their highest. In March, when normal roots had maximum food, one root of a little-leaf tree had 24 per cent reserve carbohydrates, while the other sampled root of this tree had 6 per cent. Such differences indicate that diseased trees may succumb root by

TABLE 2.—Reserve carbohydrate as a percentage of the oven-dry weight

		North Carolina	arolina					Alabama	ama			
		Healthy	thy			Hea	Healthy			Little leaf	leaf	
Month	Root	Root	Stem	Stem	Root	Root	Stem bark	Stem	Root bark	Root	Stem bark	Stem
		er cent of	er cent of oven-dry wt.		<u> </u>	er cent of	Per cent of oven-dry wt.	ند	Д	er cent of	Per cent of oven-dry wt.	•
October October Ocember Ocember February March April April Tune Full August Average	12.1 21.6 21.6 21.6 22.0 28.0 28.0 26.9 25.7 24.2 24.2	7.7 8.7.7 10.3 11.4 10.1 10.1 10.1 6.0 6.0	6.7 8.9 111.6 112.3 122.8 122.0 122.0 110.0 10.4 10.9	0.00 0.10 0.10 0.00 0.00 0.00 0.00 0.00	18.1 16.2 31.9 31.9 33.2 33.2 33.2 49.4 10.0 24.6	12 C 4 9 8 9 C C C 9 12 E C E E E E E E E E E E E E E E E E E	7.8 11.2 10.4 10.4 10.6 10.6 10.6 11.3 11.3 11.3 11.3 11.3 11.3	8.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4.4	15.6 13.6 13.6 13.6 11.0 11.0 15.7 11.8 13.6	10 10 10 10 10 10 10 10 10 10 10 10 10 1	10.8 12.8 12.8 14.3 14.6 11.3 12.4 12.2	4.0.1.0.0.1.0.0.1.0.0.1.0.0.1.0.0.1.0.0.1.0.0.0.1.0

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root, rather than by uniform decline of the root system. If this is so, there exists the implication of pathogenic action rather than unfavorable soil conditions. The variability between the two trees cut at each period averaged 27 per cent for healthy trees and 64 per cent for diseased trees, and this difference was highly significant, statistically. This high variability from tree to tree for diseased trees was due to unequal severity of the disease, and accounts for the irregularity of the cyclical graph for diseased trees in figure 1.

5. The seasonal effects in root bark and wood were similar and were more pronounced than in the stem. Seasonal effects for stem bark and wood were similar to each other, were more irregular than for corresponding root tissues, and the general level was lower.

The importance of keeping in mind the plant and plant organ in question can be illustrated by a few examples. Aldous (1) found that in the stems of sumae (*Rhus glabra* L.) and buckbrush (*Symphoricarpos orbiculatus* Moench) the least starch appeared while the plants were in flower and the maximum of starch was regained by about August 15. The shortleaf pine stem and root in the present work (Fig. 1) approached a minimum of food reserves in mid-August. Traub (17) found that the hexosans disappeared almost entirely from the cortex of apple twigs during the winter, and most observers have noted a depletion of starch in winter in stems and twigs of deciduous plants. In the shortleaf pine, however, the food reserves of the stem held constant all winter, whereas the root reserves, mostly starch, increased steadily and markedly all winter, reaching a peak in early spring (Fig. 1).

Reserve Food Content of Stem, Root, and Foliage

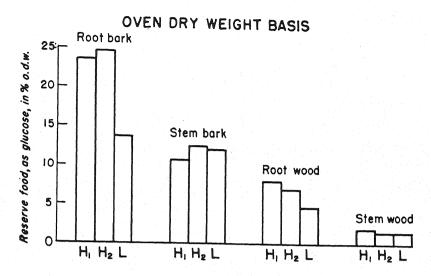
Having in mind the profound effect of season on carbohydrate content of roots, we can now consider the average amount of stored food for the different tissues for the year as a whole. Figure 2 presents these averages for stem and root, and is based upon the data that were used to construct the seasonal curves.

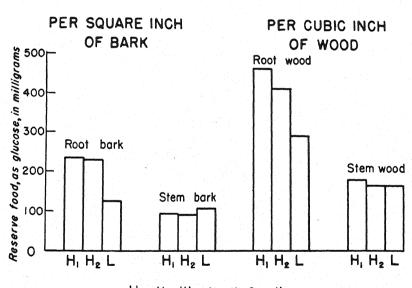
The roots of healthy trees, both bark and wood, had roughly twice as much food per unit area or volume, as roots of little-leaf trees. The roots of little-leaf trees at times were almost completely devoid of food.

The stems of healthy trees, both bark and wood, had no more food than the stems of diseased trees on either unit basis used.

In healthy trees, root bark had about twice as much food as stem bark, on either unit weight or unit area basis. Root bark had about three times as much food as root wood, and seven times as much as stem wood, on a unit-weight basis. In the average normal root 1 inch in diameter, 68 per cent of the reserve food is in the bark. The root bark is thus a tissue of great storage capacity.

In interpreting the bark data in figures 1 and 2, and table 2, consideration must be given to the fact that a fraction of the carbohydrates extracted and converted to glucose in the analysis was probably not reserve food. As





H₁ - Healthy, North Carolina H₂- Healthy, Alabama

L - Little leaf, Alabama

Fig. 2. A, average reserve food content of shortleaf pine stems and roots, for the year, as a percentage of the oven-dry weight; B, converted to milligrams of glucose per square inch of bark and per cubic inch of wood.

already mentioned, functionless outer bark, even when cleared of tannins and other coloring materials, tested about 4 per cent glucose. If we correct for this 4 per cent unavailable carbohydrate, in the bark histograms of figure 2, A, we find that root bark has nearer two and one-half times the reserve food of stem bark over the year as a whole, per unit weight.

Conditions did not permit so exhaustive a study of the carbohydrate content of other organs as was given the root and stem. In April, 1943, however, foliage and roots were analyzed from two healthy trees and two trees in advanced little leaf, near Pickens, South Carolina, to determine the relation of the amount of elaborated food in the foliage to the amount in the roots, between and within little-leaf and healthy trees. The investigation of this phase was also stimulated by the findings of Haas (6), White-Stevens (18), and others, to the effect that boron deficiency induced a high carbohydrate content in foliage and a low content in the roots. Some indications of benefit to little-leaf trees by additions of boron made this of particular interest.

The root bark of the two healthy South Carolina trees had 303 mg. of reserve food per square inch (33 per cent on oven-dry weight basis), comparing very favorably with healthy North Carolina (30 per cent) and Ala-

TABLE 3.—The reserve food content of needles and root bark of little-leaf and healthy trees in April, 1943

Carbohydrate content of tissues	Healthy trees	Little- leaf trees	Per- centage reduction
Leaves			
Carbohydrates, as per cent of oven-dry weight	16.6	11.5	31
Carbohydrates, as per cent of fresh weight	7.3	4.7	36
Carbohydrates, as mg. per linear inch	1.01	0.62	39
Carbohydrates, as mg. per needle	2.69	1.10	59
Root bark			
Carbohydrates, as per cent of oven-dry weight	32.9	11.8	64
Carbohydrates, as mg. per square inch	303.0	101.0	67

bama (33 per cent) trees for April of the preceding year (Fig. 1). The two diseased trees had an average of 101 mg. per square inch (12 per cent). From each of these trees 1,000 needles were removed by stripping several random twigs completely. The fascicles were removed, and the needles were measured and their fresh and oven-dry weights (at 75° C.) computed. Two-gram samples were used as in the other analyses. Table 3 presents the needle and root data for these trees.

The portion of the healthy twigs that bore needles (usually 2 years' growth) averaged, in length 4.4 times the needle-bearing twig length of the diseased trees. Buchanan (2) also found, for needle-bearing twig length, a ratio of about 4 to 1 for healthy compared with advanced little leaf. Table 3 shows that the average needle of a little-leaf tree contained only 41 per cent as much food as that of a healthy tree. Since there are only about one-fourth as many needles on the living twigs of diseased trees, the total foliage food content of the diseased trees is only about 10 per cent of normal. Since trees in advanced little leaf usually have 30 or 40 per cent of their twigs dead, we finally arrive at a food content of the green parts of trees in advanced little leaf of 3 or 4 per cent that of healthy trees. From this very great reduction in produced food we find good reason for the extremely nar-

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row annual rings formed by trees in the advanced stage of little leaf, for the very low food content of the roots, at times reaching almost complete exhaustion, and for the other symptoms of decline discussed by Buchanan (2).

An extra analysis of carbohydrates in needles 1 and 2 years old on healthy trees showed the 2-year-old needles to have 95 per cent as much as the 1-year-old. Although 2-year-old needles had about as much total carbohydrates as the current year's needles, in April, there still could be a question as to whether they synthesize food at the same rate as the current year's needles.

DISCUSSION

The carbohydrate cycle for roots and stems of normal shortleaf pine, as brought out in figure 1, is very different from the cycle for hardwoods, as proposed by various authors already discussed. The varying conclusions of other authors have depended to a large extent not only upon the type of plant and the organ involved, but also upon which substances they considered reserve foods. Certainly the cycle of food reserves would be quite different depending upon whether one included, in addition to starch and sugar, in the total reserves, hemicellulose, fats, pentosans, or other substances. In the present work the amount of substances hydrolyzed by takadiastase varied directly with the vigor of the trees, and it would thus appear that the amount of these substances (largely starch) comprises a good index of the amount of available food.

The present work substantiates certain work of Sablon (12) on deciduous trees, concerning which he states that stems have less reserves than roots, and that stem reserves vary much less than root reserves, usually having a lower maximum and a higher minimum than root reserves.

Buchanan (2) has described in detail the slow decline of the aerial parts of little-leaf trees, and Siggers and Doak (15) and Jackson (9) have described deteriorated roots, with dead root ends and excessive exfoliation of vital cortical tissue. The present work demonstrates that a marked reduction in carbohydrate synthesis accompanies the external symptoms of little leaf, to the extent that the reserve food production of the foliage as a whole of trees in advanced little leaf, is less than 10 per cent of normal. Since no evidence has been found of any pathogenic organisms in the aerial parts of diseased trees (9), it seems very likely that the disease either originates below the ground line as a pathogenic or physiogenic disturbance, or that it is a virus disease.

That the roots of diseased trees should be deficient in food whereas the trunks have essentially normal amounts suggests that, under conditions of insufficient food production, either the trunk retains its requirements at the expense of the roots, or initial root deficiency is due to excessive brown patch destroying the capacity of the root to store food. The latter theory seems less likely because some of the little-leaf roots analyzed had very little brown patch and yet were very deficient in food. Starvation of roots would be expected to result in the cork cambium cutting deeply into the phloem food

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storage region. We may therefore regard the excessive exfoliation of cortical tissue in little-leaf roots as a result of the disease, and thus as a symptom, rather than as a cause. The loss of this vital tissue, however, means that if recovery from little leaf could be effected, there would, for a few years, be inadequate storage tissue in the roots to accommodate normal amounts of starch. Rapid improvement in the appearance of the trees, therefore, could hardly be expected until sufficient new root phloem was formed, even where successful treatments were in operation.

Curtis (3) has concluded, from extensive experiments and review, that carbohydrates translocated to the trunk or root are used close to where they are stored, and do not come up again to be used in shoot growth. If we accept this view, then the low food reserves of little-leaf tree roots will be reflected largely by the roots themselves. The dead root ends common on such roots, as described by Jackson (9) and Siggers and Doak (15), may be the result of this root starvation, or the death of the fine roots may be a pathogenic condition that initiates little leaf. The many possible causal factors of the abnormality are being investigated by other workers.

SUMMARY

Quantitative analyses were made of food reserves in the bark and wood of normal shortleaf pines and of trees with little-leaf disease. The analyses were made throughout one year for roots and trunks and at one period in the spring for foliage.

The roots of normal trees had their minimum of reserve food in the fall. The amount increased all winter, reaching a maximum that was approximately three times the minimum, in early spring.

The stems of normal trees reached a maximum between April and June, and maintained a more or less constant lower level from July to March.

In normal trees root bark had an average for the year of about twice as much reserve food as stem bark, on either unit weight or unit area basis, about three times as much as root wood, and seven times as much as stem wood, on a unit dry-weight basis.

In a section of normal root 1 inch in diameter, 68 per cent of the reserves are in the bark, on an average basis for the year.

The root bark of little-leaf trees had an average of less than half as much food as that of healthy trees, and at times reserve food was almost entirely absent from some roots of diseased trees. The stems of little-leaf trees had as much food as normal trees at all times.

The average needle of little-leaf trees cut in April contained only 41 per cent as much elaborated food as the average needle of adjacent healthy trees. The total foliated parts of these trees were computed to have a reserve food content less than 10 per cent of normal. Two roots from each of the same trees used in the foliage analyses showed the diseased to have a reserve food content of only 33 per cent of normal.

The symptoms of decline in little-leaf trees appear to be due to a low synthesis of carbohydrates. The reason for the low synthesis is yet to be

The roots, which normally store much more food than the stems on a unit weight basis, and which fluctuate more in amount, seasonally, suffer most from inadequate nutrition. A striking effect of this root starvation is accentuated formation of rough bark owing to the cork cambium cutting deeply into the food storage region of the phloem. Thus less storage tissue remains in the root bark, and less food has to be accommodated due to reduced synthesis. The excessive storage tissue is apparently cut off by cork cambium as it deteriorates as a result of starvation.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY, Soils, and Agricultural Engineering. Agricultural RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE, in cooperation with

APPALACHIAN FOREST EXPERIMENT STATION, ASHEVILLE, NORTH CAROLINA.

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FURTHER TESTING OF COPPER FUNGICIDES FOR CONTROL OF TOMATO BLIGHT IN SOUTHWEST VIRGINIA¹

R. G. HENDERSON²

(Accepted for publication September 20, 1944)

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Experiments by the author (2) in southwest Virginia during 1940 to 1942 inclusive showed that tribasic copper sulphate and cuprous oxide, applied either as a spray or as a dust, effectively controlled early blight (Alternaria solani (E. & M.) Jones and Grout), late blight (Phytophthora infestans (Mont.) de By.), and leaf spot (Septoria lycopersici Speg.) of tomato, and thereby brought about a considerable increase in the yield of marketable fruits. Dusts were about as effective as sprays in controlling these diseases. These results, together with the opinion that dusts are more practicable to use, because they are easier to apply, led to more precise comparison of effectiveness of cuprous oxide and tribasic copper sulphate applied as dusts. Because McNew (5) indicated that increases in yield resulting from fungicidal applications were much larger on plots receiving well-balanced fertilization, the experiment included tests with side applications of nitrate of soda. The two fungicides were compared mainly under field conditions, but there were supplemental studies in greenhouse and laboratory.

FIELD STUDIES

The objective of the field studies was twofold: (1) to determine more precisely the relative efficacy of cuprous oxide and tribasic copper sulphate dusts in controlling tomato diseases, and (2) to determine whether the benefit from the use of these materials as expressed in yield of marketable fruit could be further increased by supplemental applications of nitrate of soda during the growing season.

Materials and Methods

Tomato seedlings of the varieties Rutgers, Marglobe, and Pritchard grown in the greenhouse were transplanted on May 28 into the field where tests had been made the previous year (2). An application of 500 pounds per acre of 2–12–8 fertilizer was made in bands shortly before transplanting. In addition, half of the plants in each fungicidal treatment plot received supplementary applications of sodium nitrate at the rate of 100 pounds per acre on July 7, 50 pounds on July 20, and 100 pounds on August 3.

The fungicidal treatments applied to each variety, in four replicates,

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were: tribasic copper sulphate dust,³ yellow cuprous oxide dust,⁴ and an untreated control. The treatments were applied by a motor-driven duster on July 14, 22, 30, and August 10, 14, 22, at the rate of 40 to 50 pounds per acre. Each plot consisted of 1/60 of an acre which was further divided in order to accommodate the fertilizer treatments; so yield records were taken on plots of 1/121 of an acre containing 32 plants. The fruits were harvested as they ripened (when yellow-red), and then were graded and weighed as described previously (2).

There was considerable rainfall during the 1943 season. A heavy rain of 4.86 inches within 48 hours after the first nitrate application probably

TABLE 1.—Summarized yields of tomatoes in 1943 from plots of approximately 1/30 acre

Treatment	Grade	Rut	gers	Prite	chard	Mar	globe	Av. o varie	
Tieatment	Grade	No nitrate	Nitrate	No nitrate	Nitrate	No nitrate	Nitrate	No nitrate	Nitrate
Tribasic dust	No. 1 No. 2 Culls Rots	Lbs. 328.5 265.5 250.5 24.5	Lbs. 280.5 266.3 279.0 40.3	Lbs. 242.3 278.8 368.5 22.5	$Lbs. \ 243.8 \ 275.0 \ 323.5 \ 26.0$	Lbs. 248.8 287.3 262.3 27.5	Lbs. 212.5 260.0 273.8 28.5	Lbs. 273.2 277.2 293.8 24.8	Lbs. 245.6 267.1 291.9 31.6
Cuprous oxide dust	No. 1 No. 2 Culls Rots	388.3 243.3 219.3 71.5	304.0 236.5 237.0 58.5	307.5 343.8 314.5 39.0	305.3 293.3 325.8 30.5	334.0 283.8 240.5 43.3	261.5 275.3 235.8 39.3	343.3 290.3 258.1 51.3	290.3 268.3 266.2 42.8
Check	No. 1 No. 2 Culls Rots	264.0 219.5 271.0 42.8	237.0 217.5 275.5 35.0	187.5 210.8 310.0 25.5	193.3 204.3 312.8 22.0	190.0 251.0 308.5 29.5	178.0 210.0 312.5 33.3	213.8 227.4 296.5 32.6	202.8 210.6 300.3 30.1
Average of all treat- ments	No. 1 No. 2 Culls Rots	326.9 242.8 246.9 46.3	273.8 240.1 263.8 44.6	245.8 277.8 331.0 28.8	247.5 257.5 320.7 26.2	257.6 274.4 270.4 33.4	217.3 248.4 274.0 33.7	276.8 265.0 282.8 36.2	246.2 248.7 286.2 34.8

leached most of the fertilizer from the soil. The total rainfall of 6.76 inches in June and 6.58 inches in July associated with high temperatures (5.5 degrees above normal in June and 0.5 degrees above in July) created conditions favorable to the development of the pathogens. Under such favorable conditions the pathogens developed abundantly on the lower leaves of the plants during June and by early July many leaves on the upper parts of the plants were infected. The lower leaves of plants on the experimental plots were practically all destroyed by July 14, the date of the first fungicidal application. The weather remained favorable for disease development until after the first fruits were picked on August 11. After this date rainfall was

 4 Å commercial dust mixture prepared by a local distributor from yellow cuprous oxide manufactured by Rohm and Haas Company. The metallic copper content was 4.1 per cent.

³ A commercial dust mixture prepared by a local distributor from tribasic copper sulphate manufactured by the Tennessee Copper Company. The metallic copper content was 6 per cent.

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extremely deficient throughout the remainder of the season and the mean temperature for August was 2.6 degrees above normal. The hot, dry weather during August probably hastened abscission of the diseased leaves. It can be safely stated that the 1943 season in the vicinity of Blacksburg was extremely favorable for the development of the tomato pathogens and relatively unfavorable for plant growth.

Results of Field Test

The yields of ripe fruits from the various plots are in table 1 and presented graphically in figure 1. An analysis of variance on the yields of

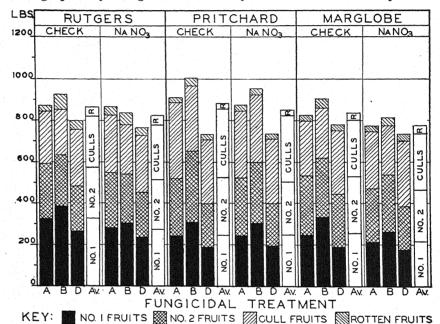


Fig. 1. Comparison of yields of fruit from the series of plots that received a basic application of fertilizer (check) with those from the series that received nitrate of soda in addition to the basic application. The unshaded column (Av.) represents the average yield for the series. The fungicidal treatments are: (A) tribasic copper sulphate dust;

(B) cuprous oxide dust; (D) no treatment.

marketable fruit (No. 1's and No. 2's combined) is summarized in table 2. The average yield of the Rutgers variety was slightly higher than either of the others, but the difference between varieties was not significant. There was no difference in the response (interaction) of the varieties to the dust treatments. The average yield of marketable fruit for the three varieties from the plots dusted with cuprous oxide was 597 pounds, for tribasic copper sulphate 532, and for the undusted check 428 pounds. The differences between these yields are highly significant; that is, the cuprous oxide treatment gave better results than the tribasic copper sulphate treatment and the tribasic copper sulphate treatment, in turn, gave better results than no treatment.

TABLE 2.—Summary of analysis of variance on pounds of marketable fruits in 1943.

Comparison	Degrees of freedom	Sum of squares	Mean squares
Blocks	3	11,328.94	
Varieties	2	1,458.25	729.13
Error (a)	6	2,253.97	375.66
Nitrogen levels	1	2,473.38	2,473.38*
Nitrogen × Varieties	2	457.53	228.77
Error (b)	9	2,740.76	304.53
Ousts:			
Cu ₂ O vs. Tribasic	1	3,152.52	3,152.52*
Dusts vs. Check	1	18,564.06	18,564.06*
Ousts × Varieties	4	1,799.42	449.86
Ousts × Nitrogen	2	441.20	220.60
Ousts × Nitrogen × Varieties	4	60.14	15.04
Error (c)	36	7,483.33	207.87
Total	71	52,213.50	
	l a company of	1	1

^{*} Significant at 1 per cent level.

The plots that received only the basic application of fertilizer gave significantly higher yields than those that received both the basic application of fertilizer and the supplementary applications of nitrate of soda; the average yields (three varieties) of marketable fruit from the two treatments being 542 pounds and 495 pounds, respectively. The first four pickings from the plots that received nitrate of soda were larger than those from the plots that received no nitrate of soda, but all the later pickings were smaller. The comparative picking yields from the Pritchard variety are illustrated

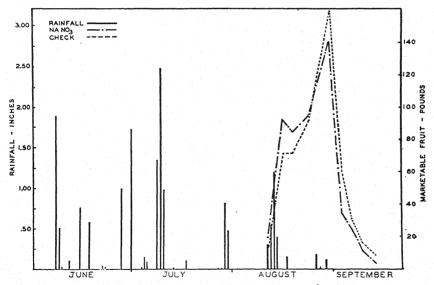


FIG. 2. Yield of marketable fruit by pickings from the Pritchard variety from 1/30 acre plots that received a basic application of fertilizer (check) as compared to the yield from plots that received nitrate of soda in addition to the basic application, and the relation of these to rainfall. The rainfall data are from the U.S. Weather Bureau records for Blacksburg, Virginia.

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in figure 2. There was no interaction between varieties and nitrate fertilization, although the difference in favor of no nitrate fertilization in the Pritchard variety was much less than that in the other varieties.

The uneven distribution of rainfall in July and August (Fig. 2) probably caused the plots side-dressed with nitrate of soda to yield less than those not side-dressed. During early August when rainfall was sufficient, the pickings of fruit from the plots side-dressed with nitrate of soda were greater than those from the plots not side-dressed, but with the onset of the drought in late August the yields from these plots became less than those from plots not side-dressed. This depression of yield by the nitrate of soda during the dry period is probably explained by Weaver and Clements' work (7) in which they found that plants side-dressed with nitrate of soda developed a shallow root system and for that reason were injured more severely by drought than plants not side-dressed with nitrate of soda.

LABORATORY AND GREENHOUSE TESTS

The results of the field test indicated that the commercial cuprous oxide dust was more toxic to the tomato foliage pathogens than tribasic copper sulphate dust as judged on the basis of increased yield of marketable fruit. But since factors other than toxicity may have influenced the field results, certain laboratory and greenhouse studies were conducted to more accurately determine the relative fungicidal efficiency of the two materials. In the laboratory, the dusts were compared on the basis of their toxicity to spores of *Alternaria solani*; and in the greenhouse, they were compared on the basis of their effectiveness in preventing infection of young tomato plants by *A. solani*.

Materials and Methods

The slide test technique outlined by the Committee on Standardization of Fungicidal Tests, American Phytopathological Society (1) for testing the toxicity of fungicidal dusts was used. The test fungus was a non-chromogenic, pathogenic, sporulating strain⁵ of Alternaria solani.

In the greenhouse tests, tomato plants of the Bonny Best variety, as recommended by McCallan and Wellman (4), were grown in a fertile potting soil in 4-inch pots and were about 8 to 10 inches high at the time the dusts were applied. Uniform lots of 5 plants each were selected. Each lot was then removed to a room adjoining the greenhouse and dusted individually.

The fungicides were applied with a plunger-type duster. Care was taken to cover the upper and lower surfaces of the leaves as uniformly as possible, but no specific quantity of dust was applied. The foliage was dry at the time it was dusted and the amount of dust that adhered to the leaves was relatively small. The plants were jarred lightly after dusting to dislodge the excess dust that fell on the more exposed leaves. After the plants were

⁵ The culture of this strain of Alternaria solani was supplied by Dr. E. K. Vaughan, by Thomas (6).

dusted, they were placed in a moist chamber for a few hours before the spore suspension of *Alternaria solani* was applied.

The moist chamber was a glass-enclosed box large enough to accommodate about 30 pots. The floor of the box was covered with wet sphagnum moss. During the day the moist chamber was protected from direct sunlight to avoid overheating. The temperature in the chamber ranged between 21° and 28° C.

The inoculum was sprayed onto the upper and lower surfaces of the leaves by means of a hand-operated atomizer. A sufficient amount of the suspension was applied to wet the surface of the leaves but not enough to run. After being atomized, the plants were returned to the moist chamber where a high relative humidity was maintained for 36 hours.

Early blight lesions were visible on the leaves of inoculated plants in about three days but the readings were not made until after the sixth or seventh day. The fourth, fifth, sixth, and seventh leaves from the base were removed from the plant and all the lesions on all the leaflets counted and recorded as the total number of lesions per plant.

Five greenhouse tests were run. In the first four, the full strength dust as received in the package was used, but in the fifth test the dusts in addition to being used full strength were diluted with talc in the ratio of one to three, one to one, and three to one. The checks consisted of a series of undusted plants and a series dusted with talc.

Results of Laboratory Tests

The laboratory data were inconclusive because of the wide variability of results from the different tests. This variability was apparently due to the cuprous oxide being difficult to wet with water, so that a portion of the toxicant floated on the surface of the drop of spore suspension, and also to the non-uniformity of samples resulting from treatment with poorly mixed tribasic copper sulphate dust. In small dosages, cuprous oxide dust appeared to be more toxic to spore germination than similar dosages of tribasic copper sulphate dust, but in large dosages, the results were inconsistent.

Results of Greenhouse Tests

The tribasic copper sulphate dust applied to tomato plants in the green-house reduced the number of infections of *Alternaria solani* from an average of 120.25 per plant, as shown by the check, to 7.05 per plant; and cuprous oxide reduced the number to 1.85 per plant (Table 3). In four trials cuprous oxide consistently gave better control than tribasic copper sulphate.

Dilutions of re-mixed tribasic copper sulphate dust with tale gave progressively less control of early blight as the dilution increased (Table 4). The one-fourth strength dust gave an average of 21.75 lesions per plant, while the one-half and three-fourths strengths gave 18.25 and 14.50 lesions, respectively. Dilution of cuprous oxide dust with tale did not give a proportionate reduction in control. The one-fourth strength gave 13.25 lesions per plant; the one-half strength, 9.25; and the three-fourths strength, 10.75.

TABLE 3.—Number of lesions developed on dusted and undusted tomato plants inoculated with Alternaria solani in 4 experiments in the greenhouse

		No. lesion	ns on each o	f 5 plants		Average
Treatment	1	2	3	4	5	per plant
Check, not dusted	104 128 42 241 128.75	67 27 29 206 82.26	83 118 60 467 182.00	115 56 75 66 78.00	96 90 102 233 130.25	93.00 83.80 61.60 242.60 120.25
Tribasic dust Average	$egin{array}{c} 16 \\ 6 \\ 3 \\ 4 \\ 7.25 \end{array}$	6 7 6 10 7.25	19 3 14 5 10.25	1 3 5 2 2.75	11 1 1 18 7.75	10.60 4.00 5.80 7.80 7.05
Cuprous oxide dust Average	3 0 1 4 2.00	2 1 5 6 3.50	5 1 0 0 1.50	2 3 3 0 2.00	0 1 0 0 0.25	2.40 1.20 1.80 2.00 1.85

DISCUSSION

Records for the past several years show that foliage diseases on unprotected tomatoes have reduced yields 30 to 40 per cent each year in southwest Virginia. Such consistent losses indicate that spraying or dusting of tomatoes in this area would be profitable each year. However, the returns from applying a fungicide depend not only on disease control but also on seasonal factors and soil fertility which determine the growth and productivity of the crop. This was clearly demonstrated in 1943 when the environmental factors of the field test were unfavorable for optimum plant growth. The basic application of fertilizer was moderately light and a number of heavy rains

TABLE 4.—Number of early blight lesions developed on tomato plants dusted with different dilutions of fungicides

Treatment	Ratio	No.	No. lesions on each of 4 plants					
Tieatment	dust: talc	1	2	3	4	per plant		
Check Talc		202 52	168 30	71 39	311 29	188.00 37.50		
Tribasic dust Average	1:3 1:1 3:1 1:0	19 11 6 10 11.50	24 12 24 15 18.75	19 36 24 2 20.25	25 14 4 1 11.00	21.75 18.25 14.50 7.00 15.38		
Cuprous oxide dust Average	1:3 1:1 3:1 1:0	5 11 6 5 6.75	13 16 17 11 14.25	16 9 17 4 11.50	19 1 3 3 6.50	13.25 9.25 10.75 5.75 9.75		

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in June and July further depleted the soil fertility through leaching of the soluble nutrients. Also, drought conditions in August and September were very injurious to the crop. Under these conditions, the increase in yield as a result of dusting was not so large as that obtained in previous years (2), but even under such adverse conditions the fruit from the dusted plants was of superior quality and there was an increase in yield of 2 or 3 tons per acre. It was obvious, too, that in 1943 the dust should have been applied at least 10 days earlier for the best disease control.

Side applications of nitrate of soda in this test depressed yields slightly, but the results might have been different had the rainfall been uniformly distributed throughout the growing season.

The tribasic copper sulphate dust used in 1943 was definitely inferior to the cuprous oxide dust in preventing the foliage diseases. This inferiority was shown in the field tests where yield was the criterion and also in the greenhouse tests where the number of infections by Alternaria solani was the criterion. A comparison of the two materials in the laboratory by the spore toxicity test indicated that cuprous oxide was more toxic to spores of A. solani than tribasic copper sulphate in low dosages but these tests were inconclusive. The poor blending of the toxicant with the diluent undoubtedly accounted in part for the poor showing made by the tribasic copper sulphate dust in the field, but the results of the one greenhouse test with a re-mixed dust indicated that a properly blended tribasic copper sulphate dust was also less effective in preventing infection by Alternaria solani than was cuprous oxide dust. The average number of early blight lesions on plants treated with the re-mixed tribasic copper sulphate dust was 15.38. while on plants treated with cuprous oxide dust the average was 9.75. This evidence is not conclusive but it indicated that the toxicity of tribasic copper sulphate to A. solani is less than that of cuprous oxide. Horsfall and Heuberger (3) showed that the more finely divided forms of copper, as cuprous oxide, were more toxic to fungi than the less finely divided forms, as tribasic copper sulphate.

The most efficient use of metallic copper in the fungicide was in the form of cuprous oxide, because the cuprous oxide dust contained only 4.1 per cent metallic copper and the tribasic copper sulphate dust 6.0 per cent. In field performance, cuprous oxide dust in 1943 was superior to the tribasic copper sulphate dust and it was at least equally as good in previous years (2). Therefore, cuprous oxide should be recommended over tribasic copper sulphate during the war period when a shortage of copper exists.

SUMMARY

Cuprous oxide and tribasic copper sulphate dusts were compared as to their efficiency in controlling early blight, late blight, and Septoria leaf spot of tomatoes in southwest Virginia on plots receiving a basic fertilization only and on plots receiving supplemental applications of nitrate of soda. The toxicities of these two dusts to spores of *Alternaria solani* were also compared

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in laboratory and greenhouse. The data obtained warrant the following conclusions:

- 1. Applications of the commercially mixed cuprous oxide dust available to growers in 1943 in southwest Virginia gave about 10 per cent higher yields than the commercially mixed tribasic copper sulphate dust.
- 2. In 1943, when August and September were abnormally dry, side applications of nitrate of soda depressed the yield of marketable fruit about 8 per cent.
- 3. The commercially mixed cuprous oxide dust was more effective than the tribasic copper sulphate dust in preventing infection of tomato foliage by Alternaria solani in the greenhouse. This difference was due in part to the toxicant in the tribasic copper sulphate dust not being sufficiently blended with the diluent.

VIRGINIA AGRICULTURAL EXPERIMENT STATION, BLACKSBURG, VIRGINIA.

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ADDITIONAL SPECIES OF LILIUM SUSCEPTIBLE TO LILY-ROSETTE VIRUS

PHILIP BRIERLEY¹ AND FLOYD F. SMITH²
(Accepted for publication September 15, 1944)

Lily rosette,³ or yellow flat in Ogilvie's original description,⁴ is the most destructive virus disease of Easter lilies (*Lilium longiflorum* Thunb.). Lily growers cannot be too strongly warned against its introduction into production areas. Presumbaly of eastern Asiatic origin,⁵ and best known from Bermuda,⁴ rosette is reported also from England, Holland, and Java. Within the United States rosette is still restricted in distribution, and has been experimentally confirmed thus far only from one planting in southern Florida.

Little information on host range of the rosette virus is available, for Ogilvie⁴ studied it in the Easter lily only. Rosette symptoms have been reported in *Lilium batemanniae* Wallace [L. dauricum var. venustum (Kunth) Wilson f. batemanniae (Wallace) Wilson] by Guterman⁶ and in L. auratum Lindl. by K. M. Smith,⁷ but neither of these writers mentions experimental transfers.

In the tests reported herein the rosette virus was originally isolated from Florida-grown Easter lilies. Transmissions were by the melon aphid, Aphis gossypii Glover, the only known vector, from naturally infected plants or from experimentally infected Easter lily seedlings. Leaves of diseased Easter lily seedlings have the characteristic downward curling 3 to 5 weeks after exposure, frequently become yellowed, and occasionally the upper leaves redden. Flowers, if produced on such plants, are noticeably smaller than normal and fail to open fully. Stem bulbils are developed freely in the lower leaf axils and occasionally in the upper leaf axils of blind plants. Early maturity is the rule. Infected bulbs are poorly developed and often decay, leaving only a cluster of small bulbils for propagation.

Virus-free seedlings of a number of species and varieties of *Lilium* were exposed to rosette by transfer of *Aphis gossypii* from affected Easter lilies. Three to 8 plants of each were inoculated, and return transfers to confirm susceptibility were made 2 to 11 months after exposure. Symptoms were

¹ Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, and ² Entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland.

³ British Mycological Society. List of common names of British plant diseases. Trans. Brit. Mycol. Soc. 14: 140-177. 1929.

4 Ogilvie, L. A transmissible virus disease of the Easter lily. Ann. Appl. Biol. 15: 540-562. 1928.

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7 Smith, K. M. The virus diseases of glasshouse and garden plants. Sci. Hort. 4: 126-140. 1936.

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Fig. 1. A. Rosette in *Lilium sargentiae* inoculated April 12, photographed October 27, 1943, showing stem bulbils produced above the point of inoculation, and curled leaves developed from these bulbils. B. Initial symptoms of rosette in the same plant shown in A, photographed 29 days after inoculation (left) in comparison with uninoculated plant (right). C. Rosette in *L. formosanum* (left) with control (right), photographed April 29, 1944, in 5-inch pots 70 days after inoculation.

evident after about 3 weeks in the species L. formosanum Stapf (Fig. 1, C), L. sargentiae Wilson (Fig. 1, A, B), and L. umbellatum Hort. In these

species the disease can be diagnosed with reasonable accuracy. For brevity the symptoms observed in experimental plants are summarized in table 1. In these tests no other species showed as well-defined symptoms as the Easter lily. Many diseased species developed only slight dwarfing, irregular curling, or reddening, which in the field might be confused with the effects of root injury or poor cultural conditions.

There was considerable variation in symptom expression within species and varieties, and also in the same plant at different seasons. Therefore, all

TABLE 1.—Species and varieties of Lilium experimentally infected with lily-rosette virus and confirmed by return transfer to Easter lily

			Sympt	omsa		
Species or variety	Plants		Leaves		Flow	ers
	Dwarfed	Curled	Yel- lowed	Red- dened	Dwarfed	Lack- ingb
L. dauricum Ker-Gawl. L. davidi Duchartre L. davidi var. willmottiae Cotton & Grove L. elegans Thunb. L. formosanum Stapf L. henryi Baker L. leucanthum Baker L. longiforum Thunb. L. myriophyllum var. superbum (Baker) Wilson L. regale Wilson L. sargentiae Wilson L. speciosum Thunb. L. umbellatum Hort.	+++++++++++++++++++++++++++++++++++++++	+ + + + + + + + + + + + + + + + + + + +	+++++++++++++++++++++++++++++++++++++++	++++++++	+ + + - + + - + + + + + + + + + + +	+ + + + + + + + + + + + + + + + + + + +

 $^{^{}a}+,++,+++$ indicate increasing intensity of the symptom; - indicates that it was not observed.

symptoms scored in table 1 are not necessarily evident in a given plant at a given time; and it is also possible that some effects scored as absent might be expressed if larger numbers of plants of these species were infected. Premature withering of leaves and of the upper stem were noted in *Lilium davidi* Duchartre and *L. leucanthum* Baker. There was no evidence of susceptibility of Clara Butt tulips to lily rosette in 5 tests (50 plants).

It is clear that lily rosette is of considerable potential importance in the case of various garden lilies. Moreover, many of these lilies, although worthless for ornament when affected, fail to show sufficiently clear symptoms to permit accurate diagnosis, and could serve as unsuspected reservoirs of the rosette virus.

BUREAU OF PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND.

b Absent from at least some of the plants in a test lot.

PATHOGENICITY OF ISOLATES OF RHIZOCTONIA SOLANI FROM POTATOES

L. H. PERSON

(Accepted for publication September 18, 1944)

While studying the pathogenicity of strains of Rhizoctonia solani on bunch snapbeans, there were included in one of the inoculation tests 11 isolates from Irish potatoes. Of these, 6 were from stem lesions and 5 from sclerotia on the tubers. None of them was pathogenic to beans. This suggested two questions, answers to which seem important. Seed potatoes coming into Louisiana frequently have on them large numbers of sclerotia of Rhizoctonia. No losses have been observed following the planting of such potatoes and consequently the treatment of tubers with fungicides that would destroy the sclerotia has never been recommended. On the other hand, many Louisiana crops are severely attacked by Rhizoctonia. questions for which solutions seemed desirable were: (1) Do the sclerotia on potato tubers brought into Louisiana for seed purposes produce strains that attack the stems of young potato plants? (2) Do these strains attack other plants in Louisiana and does the planting of tubers with sclerotia add undesirable pathogenic strains to the soil? To answer these questions a rather large number of isolates obtained from sclerotia on tubers and from lesions on potato stems were tested on bean and potato stems in comparison with isolates from other hosts.

The isolates used included 97 cultures from sclerotia on Irish potato tubers from 71 cars of seed potatoes shipped into Louisiana in 1941 from Nebraska, North Dakota, Maine, and Colorado; 4 cultures from sclerotia on tubers raised in Louisiana; 3 cultures from Dr. Kotila who isolated them from sclerotia on tubers; 29 cultures from stem lesions on Irish potato plants in Louisiana; 2 cultures from beans; and 2 cultures from sugarbeets which had been found pathogenic in previous tests.

In the inoculation tests with beans the plants were grown in sterilized soil, inoculated with the cultures of *Rhizoctonia*, and the severity of infection on the stems was noted at the end of about 20 days. For the inoculation tests with Irish potatoes, clean tubers were sterilized for 5 minutes in acidified bichloride of mercury. These were planted in sterilized soil and the inoculum was then added. Pathogenicity was measured by the size of the lesions on the young stems. With beans two tests of two replications of 20 seeds each were made with each culture, except for a few cultures that were tested only once. In most instances the results with Irish potatoes represent the average of 3 tests, each with 8 or more plants.

The results of inoculating beans are in table 1. Only 3 of the 101 cultures from sclerotia from potato tubers attacked beans and these were only slightly pathogenic. Of the 29 isolates obtained from lesions on potato stems, 9 were strongly pathogenic, causing deep lesions on the bean stems,

TABLE 1.—Relative pathogenicity on beans of 101 cultures from sclerotia on Irish potatoes and 29 cultures from lesions on potato stems^a

3° 1	Number of cultures						
Source of cultures	Non- pathogenic	Slightly pathogenic	Strongly pathogenic	Total			
Sclerotial isolates Colorado Louisiana Maine Michigan Nebraska North Dakota Unknown Total Stem lesion isolates Louisiana	5 4 5 1 58 25 98	""" ""2 "1 3	9	5 4 5 1 60 25 1 101			

^a Two isolates from bean stem lesions and 2 isolates from sugarbeets included as check cultures in these tests were strongly pathogenic to beans.

one was slightly pathogenic, and 19 were nonpathogenic. The 2 bean isolates and 2 sugar-beet isolates used as check cultures were strongly pathogenic in the tests. The control plants were not infected.

In table 2 are the results of the tests with young potato plants. Seventeen of the 70 isolates from potatoes were not pathogenic, 35 were slightly pathogenic, 13 were moderately pathogenic, and 5 were strongly pathogenic. The 2 cultures from beans and the 2 from sugar beets used as checks were moderately pathogenic to potato plants. The control pots were free of infection.

Of the 3 cultures from sclerotia on tubers which had been pathogenic to beans, one was not pathogenic to Irish potatoes and two were only slightly pathogenic.

It would seem from these studies that the sclerotia present on Irish potato tubers have little effect on the Rhizoctonia solani potential in the soil. On the other hand a number of isolates from potato stem lesions were patho-

TABLE 2.—Relative pathogenicity of 70 cultures of Rhizoctonia isolated from sclerotia on Irish potato tubersa

		Nu	mber of cultur	es	
Source of cultures	Non- pathogenic	Slightly pathogenic	Moderately pathogenic	Strongly pathogenic	Total
Colorado Louisiana Maine Michigan Nebraska North Dakota Unknown Total	1 1 1 9 4 1	1 2 5 15 12 35	1 1 9 2 13	4 1	3 3 6 1 37 19 1

^a Two isolates from bean stem lesions and 2 isolates from sugar beets included as check cultures in these tests were moderately pathogenic to Irish potatoes.

genic to beans, and it is possible that a potato crop preceding beans may have some effect on the perpetuation or increase of strains of the fungus pathogenic to beans. It is probable that the stem lesions from which these isolates were obtained resulted from infection with soil *Rhizoctonias* and did not come from sclerotia or mycelium on the seed tubers.

The fact that 70 per cent of the sclerotial isolates were either non-pathogenic or only slightly pathogenic to Irish potatoes and only 7 per cent were strongly pathogenic indicates that most of the sclerotia on the tubers play relatively little part in increasing the damage done by *Rhizoctonia solani* to Irish potatoes. These results agree with those of Sanford¹ who showed that the majority of cultures from sclerotia were nonpathogenic or of marginal pathogenicity. This may help to explain the erratic results obtained by many investigators in seed treatment tests on potato tubers for the control of *R. solani*.

DEPARTMENT OF PLANT PATHOLOGY,

LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE, LOUISIANA.

¹ Sanford, G. B. Studies on *Rhizoctonia solani* Kühn. III. Racial differences in pathogenicity. Canad. Jour. Res. (C) 16: 53-64. 1938.

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LEAF SCALD OF SUGAR CANE IN BRAZIL

S. C. ARRUDA AND J. FRANCO DO AMARAL (Accepted for publication September 30, 1944)

Since March, 1943, studies have been in progress on a disease of sugar cane which has been present for several years at the Estacão Experimental de Cana de Açucar and on neighboring sugar-cane properties in Piracicaba, State of São Paulo, Brazil. The disease causes an intense chlorosis of the top leaves of the affected plants and has therefore been referred to as sugar-cane albinism. Preliminary investigations showed that the disease was transmitted from the seed pieces and that every bud of a diseased stalk invariably gave rise to a diseased plant.

Field and greenhouse observations of affected plants have led us to conclude that the albino condition of the top leaves is only a final stage in the development of the disease, and that in the primary stage there are less conspicuous chlorotic streaks with sharply-defined borders, 1-2 mm. wide, which run from the tip to the base of the leaf and continue down on the leaf sheath. Later, as the streaks broaden from the tip downward, there is a progessive withering of the tissues in the same direction, giving a final scalded appearance to the leaves. The severely affected stalks very commonly produce lateral shoots and on these the developing leaves very quickly show the typical chlorotic streaks. In the more advanced stages of the disease, especially on the more susceptible varieties, the top leaves on the affected stalks become completely chlorotic. When a diseased stalk that is approaching maturity is split and examined, a light red discoloration of the fibrovascular bundles, mainly in the nodal regions, is frequently observed. This discoloration seems to be more pronounced in susceptible varieties such as C.P. 29/320.

A comparison of the symptoms with descriptions of other sugar-cane diseases not known to occur in Brazil has shown that the Brazilian disease agrees very closely with what is found in the literature for the chronic phase of leaf scald, a bacterial disease caused by *Bacterium albilineans* Ashby. The identity of the disease has been confirmed by microscopic examinations and by cultures. Leaf scald is distributed in the Eastern Hemisphere and Hawaii, but up to this time has not been reported on the mainland of the Western Hemisphere.

Microscopical examinations have shown that the bacteria are present in the xylem elements of the fibrovascular bundles in the affected stalks as well as in the leaf streaks. Pure cultures have been obtained by first making tissue plantings in a 25 per cent sugar-cane juice and peptone medium in tubes and then making dilution plates after about 8 days from those tubes that became slightly turbid. An incubation temperature of 25° C. was used.

The cultural behavior and the physiological and morphological characters of the Brazilian organism agree closely with those of *Phytomonas albi-*

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lineans Ashby as they have been presented by Martin, Carpenter and Weller, Wilbrink, and North.

The bacterium is a slender, rod-shaped organism, Gram-negative, forming chains and threads in culture but not producing spores. It is motile, though the flagellae have not as yet been successfully stained by Zettnof's method. In agar plates (after 5-8 days), it forms small to minute colonies with glistening surface and entire margins, viscid and buff yellow. Broth becomes turbid and a filament forms which is fixed to the bottom of the tube. Other physiological reactions include: fair growth in sugar but no fermentation; fair growth in peptone water in presence of carbohydrates; no hydrolysis of starch; hydrogen sulphide, indol, and ammonia production negative; no reduction of nitrates; no liquefaction of gelatin; no change

TABLE 1.-Inoculation tests with the bacterium associated with the albinism of sugar cane in Brazil

Culture	Origin of culture	Inoculation date	Seed pla:	pieces nted	info aft	ants ected er 2 nths	Infection
			Inoc.	Check	Inoc.	Check	
27 48 49 55 77	C.P. 29/320 P.O.J. 2878 C.P. 29/320 Co. 281 Co. 290	Apr. 20, 1944 Aug. 5, 1943 do Oct. 29, 1943	No. 31 21 14 14 10	No. 15 9 5 6 5	No. 16 2 6 10 10	No. 0 0 0 0 0 0 0 0	Per cent 51.6 9.5 42.8 71.4 100.0

To prove that the bacterial cultures isolated from affected cane plants were pathogenic and would produce the symptoms observed in the fields, seed pieces were inoculated under controlled conditions with four cultures, Nos. 27, 48, 49, and 55. Cultures were isolated from stalks of different varieties from several places in Piracicaba, the first in March and the others in July, 1943. Single-eye seed pieces were selected. The inocula which consisted of suspensions of bacteria from pure cultures in sterile water were smeared on the cut ends of the seed pieces just before planting. Seed pieces of the variety Co. 290 were used although it is not the most desirable variety for inoculation tests due to its relative resistance or tolerance to the disease. The results from the inoculation tests are in table 1.

In the tests, the shoots growing from seed pieces inoculated with cultures 48, 49, and 55 came up in an apparently healthy condition. One month after the inoculations were made, however, a withering of the tips of the basal leaves was noted. This withering progressed downward to the bases

¹ Martin, J. P., C. W. Carpenter and D. M. Weller. Leaf scald disease of sugar cane in Hawaii. Hawaiian Planters' Rec. 36: 145-196. 1932.

² Wilbrink, G. De gomziekte van het suikerriet, hare oorzaak en hare bestrijding.

3 North, D. C. Leaf scald, a bacterial disease of sugar cane. Colonial Sugar Refining Co., Ltd., Sidney, N. S. W. Agric. Rept. No. 8. 1926.

of the leaves and the other portions of the plants appeared much paler than the control plants. Some of the plants wilted and died, appearing to have the acute stage of the disease. Most of the plants, however, had the general appearance of recovering to a certain extent with the disease perhaps in a dormant condition for a period of 1 or 2 months. After that period, the typical chlorotic streaks, characteristic of the disease, were observed. The inoculations with culture 27 were made during cool weather and the shoots took a longer time to come up. Several of these appeared above the ground in a completely chlorotic condition, though most of them did not become completely chlorotic for about 5 months.

The bacterium was reisolated from Co. 290 plants inoculated with culture 55 and infection was obtained when one of the isolates (culture 77) was inoculated on C.P. 29/291. (See table 1.)

As the symptoms of the Brazilian disease agree with those described for leaf scald and as the morphological, physiological, and cultural characters of the causal organism agree with those of *Phytomonas albilineans* Ashby, it is believed that the evidence is sufficient to consider that the Brazilian disease is identical with the leaf scald of the Eastern Hemisphere.

Instituto Biológico, São Paulo, Brazil.

PHYTOPATHOLOGICAL NOTES

Phytophthora Wilt and Stem Canker of Cinchona.—Seedling blights of cinchona, caused by Phytophthora spp., have plagued growers in many parts of the eastern hemisphere. Observations by growers indicate that similar diseases will be of economic importance in the establishment of a cinchona bark industry in the American tropics.

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Celino, in 1934, reported that Phytophthora faberi Maubl. (synonymous with P. palmivora Butl.2) was causing a severe blight of seedlings of Uinchona calisaya var. ledgeriana Howard, C. hybrida Hort., and C. succirubra Pavon in the Philippines. Celino considered the disease an important factor in the growing of cinchona. In 1935 Kheswalla3 reported a similar serious disease, caused by P. palmivora, on seedling C. ledgeriana Moens in Sawada,4 in 1936, reported a disease in Formosa on cinchona seedlings with symptoms differing slightly from those reported from the Philippines and India. As described by him, the infection apparently occurred on the stem with the Phytophthora subsequently spreading upward to the tip of the plant. In India and the Philippines the infection court was apparently the tip of the plant. The Formosan Phytophthora was considered a new species and was described as P. cinchonae Saw. Seedling blights of cinchona caused by Phytophthora have not been reported from Java, although Hartley⁵ reported P. faberi (P. palmivora) on other hosts. Hartley stated orally that the forms of P. palmivora he studied from several hosts in Java differed from most of the isolates of P. palmivora that he had observed from other parts of the world. One outstanding difference was the sparse sporulation of the Java forms. They nevertheless caused similar disease symptoms and probably were referable to P. palmivora.

As reported in the literature, the Phytophthora diseases mentioned above apparently affect seedlings only. However, a disease or diseases with similar symptoms were observed by the authors in Central and South America on seedlings, grafted stock, and mature and semi-mature plantation trees. A similar disease was also observed in Puerto Rico on seedlings only. The infection court seems to be the petiole region of the tender new leaves or the succulent stem tissue. In the early stages on the leaves the fungus causes brown, necrotic spots with indefinite margins. From initial infections at the base of one or more leaf blades the fungus may grow through the entire length of the petiole and enter the stem. On seedlings the entire tree is killed as the pathogen spreads downward through the cambial region of the stem.

¹ Celino, M. S. Blight of cinchona seedlings. Philippine Agr. 23: 111-127. 1934. ² Tucker, C. M. Taxonomy of the genus Phytophthora de Bary. Mo. Agr. Exp.

Stat. Res. Bull. 153. 1931.

Stat. Res. Bull. 153. Seedling blight of Cinchona ledgeriana Moens caused by Phytophthora palmivora Butl. in the Darjeeling district. Indian Jour. Agr. Sci. 5: 485-495.

⁴ Sawada, K. [Phytophthora blight of cinchona seedlings occurring in Formosa, caused by Phytophthora cinchonae Saw. n. sp.] Formosan Agr. Rev. 32: 326-346. 1936. 5 Hartley, Carl. [Diseases of Cacao.] Instituut voor Plantenziekten, Dept. van Landbouw, Nijverheid en Handel Bull. 19. 1924.

On older grafted stock and seedling plantation trees the symptoms of infection are elongated, slightly sunken cankers and dieback of the more succulent branches. In some cases on older trees the terminal portion of every branch is killed, leaving the tree with clusters of brownish-black dead leaves.

This disease was observed in Guatemala at several locations on seedling Cinchona pubescens Vahl (called C. succirubra locally), C. officinalis L., and the Ledger form of C. officinalis. It was also noted on the Ledger form grafted on C. pubescens in nursery and plantations and on C. officinalis and C. pubescens in plantations. The disease was observed in Puerto Rico on nursery stock of the Ledger form of C. officinalis. Stem cankers on both C. pubescens and C. pitayensis Wedd. and branch dieback on C. pubescens

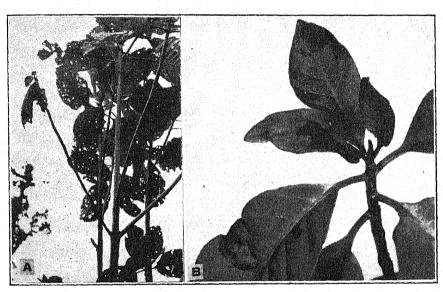


Fig. 1. A. Dieback of branch tip of Cinchona pubescens in Colombia. B. Tip dieback and infection of adjacent leaves induced by inoculation on the Ledger variety of C. officinalis.

were observed in Colombia on cinchona trees growing wild in forest stands (Fig. 1, A).

In Peru, seedlings of the Ledger form of C. officinalis and some cinchona hybrids as well as plantation stock of the Ledger form were diseased with symptoms similar to those observed in Guatemala, Colombia, and Puerto Rico. From part of the Peruvian seedling material a Phytophthora, tentatively identified as P. parasitica Dast., was isolated from leaf blade, petiole, and stem material. This is apparently the first recorded occurrence of this species on cinchona as well as the first reported Phytophthora blight of cinchona in the western hemisphere. Two isolates available for study were characterized by optimum growth temperatures of 25° C. but were able to grow at temperatures as high as 37.5° C. Eight-day-old cultures on cornmeal agar produced prominently papillate sporangia, averaging $30 \times 24 \,\mu$, on branching sporangiophores 2 days after the plates were flooded with water.

The pathogenicity of one of the 2 isolates has been demonstrated by inoculation on 18-inch potted plants of the Ledger form of C. officinalis grown from seed of Philippine origin. Six days after inoculation, 3 of 4 stem inoculations with mycelium introduced into a small cut to the cambium had produced girdling cankers, while 2 check incisions in which sterile agar was introduced remained healthy. Four bud tips inoculated without wounding by placing mycelium in agar blocks in contact with them became infected. and dieback was produced (Fig. 1, B); 2 check treatments remained healthy. Twelve mature, unwounded leaves were inoculated by placing mycelium growing in agar blocks in contact with the upper surface. Five infections resulted with symptoms typical of leaf infection as it occurred in the field. Infections did not spread through the petiole to the stem, however, because the leaves dropped before this occurred. Six leaves on which sterile agar was placed remained uninfected. The fungus was recovered from one of the 5 leaf infections, 2 of the 4 tip infections, and at a distance of $\frac{1}{2}$ inch or more from the point of inoculation from all 4 of the stem cankers. One of the 4 stem inoculations did not result in a girdling canker. The tree, however, suddenly wilted and lost its leaves 11 days after inoculation. The canker had not girdled the stem, although the *Phytophthora* was still alive and was recovered from the margin of the infection. Dissection and culturing of the entire tree demonstrated that the cortical tissue from root to tip was infected. At the time of culturing only slight discoloration gave indication of this infection.

Some control apparently was obtained in Peru by eradicating wilted seedlings from the beds. In Guatemala, control also was secured and considerable material salvaged by cutting back the tops of infected grafted stock to well below the dead tip. The cortical infection that resulted in the single inoculated tree might give indication that cutting back, unless done very early and severely, may not give control. Experience with P. parasitica in the United States would seem to indicate that control could be obtained by regular application of a copper spray containing a good sticker. the disease was observed, considerable evidence of host resistance of individuals and of certain clonal lines was apparent. It appears likely that the selection of resistant clones of high alkaloid content will be possible.—Bowen S. CRANDALL, Associate Pathologist, and WILLIAM C. DAVIS, Pathologist. Division of Latin American Agriculture, Office of Foreign Agricultural Relations, in cooperation with Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

Crater Rot and Blotch of Celery, a New Aspect of Soft Rot Caused by Erwinia carotovora.—Erwinia carotovora has been found to produce conspicuous and destructive symptoms other than heart rot which result in large losses in commercial celery fields in the Delta region in California. Small spots, at first watery, then straw-colored to brownish, appear near the base of

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the petioles (Fig. 1, B), and later become sunken, dark-brown, with sharply defined edges (Fig. 1, D). In some cases only superficial lesions are induced (Fig. 1, C), resulting in a blotchy appearance, in contradistinction to the deeper, soft watery rot. Later, the affected tissues dry out and collapse resulting in concave, erater-like depressions.

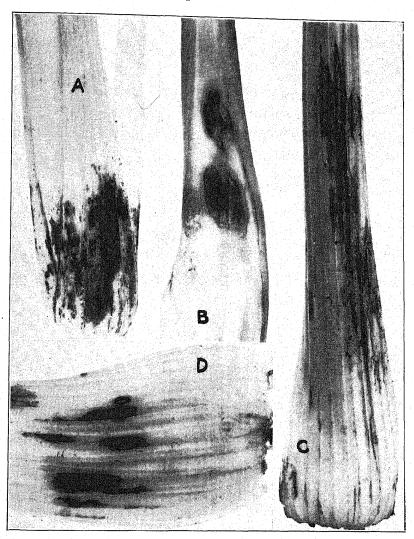


Fig. 1. A. Crater rot and blotch of celery caused by *Erwinia carotovora* on Golden Self Bleaching celery grown in peat soil, before ridging. B. Crater rot on ventral side of a petiole. Golden Self Bleaching variety. Plants were ridged and heavily irrigated. C. Blotch disease of celery (*E. carotovora*) on dorsal side of a petiole of Golden Self Bleaching celery, from plants that were not ridged. D. Dorsal side of a petiole of Golden Self Bleaching celery showing crater rot. Collapse of the tissues caused deeply sunken depressions with sharp edges. Celery was grown in peat soil and heavily irrigated.

In some years losses from crater rot and blotch are severe, since badly diseased plants cannot be shipped and stripping off of the outer larger peti-

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oles is necessary. The practice of leaving diseased stalks in the field results in heavy infestation of the soil with the crater-rot bacteria and may account for the increase of the disease when celery is repeatedly grown in the same field.—P. A. Ark, University of California, Berkeley 4, California.

Susceptibility to Black Root Rot of Apple Trees Having Various Root and Top Combinations.—In continuation of previously reported work on the susceptibility of own-rooted trees to black root rot¹ another experiment is here reported where trees having various root and top combinations were tested for their susceptibility to the black-root-rot fungus (Xylaria mali Fromme).

TABLE 1.—Percentages of roots of various apple varieties infected by Xylaria mali, averages for four seasons

Root variety	Cortex infection only	Deep infection	Total infection	Av. size of lesion
	Per cent	Per cent	Per cent	mm.
Arkansas	12.5	83.3	95.8	29.6
Ben Davis	41.7	56.2	97.9	28.6
Delicious	11.4	74.4	85.8	32.3
Fallawater	17.1	72.0	89.1	32.4
McIntosh	9.2	84.4	93.6	26.6
Nero	30.1	61.6	91.7	25.7
Northern Spy	11.6	74.2	85.8	32.4
Opalescent	9.9	72.8	82.7	25.9
Perkins	16.6	66.7	83.3	35.7
Red Astrachan	12.5	75.0	87.5	22.0
Rome Beauty	15.9	67.2	83.1	24.4
Smith Cider	16.5	70.5	87.0	30.1
Starking	22.5	77.5	100.0	34.7
Stayman Winesap	25.3	66.4	91.7	29.7
Summer Rambo	26.4	67.4	93.8	27.0
Wealthy	36.4	58.2	94.6	28.1
Williams	32.7	55.3	88.0	26.8
Winesap	25.0	68.7	93.7	32.3
Winter Banana	12.5	75.0	87.5	19.7
Yellow Transparent	17.4	69.3	86.7	25.2
York Imperial	22.6	63.6	86.2	26.1

a All figures represent averages for 1938 through 1941.

In 1937 apple trees 2 years from the graft were set 18 inches apart in rows 4 feet wide. Inoculation was begun in 1937 and continued each year for 4 years.

The trees were either own-rooted or consisted of varieties worked on an own-rooted tree, so that top and root were of known varieties. A separate record of the percentage of infection and size of lesion was made for each root and top combination. Since there were no outstanding differences in susceptibility because of top influence, the various root and top combinations were grouped according to root variety, of which there were 21, as given in

¹ Cooley, J. S. Factors affecting distribution and severity of black root rot of apple trees. Jour. Agr. Res. [U.S.] 65: 299-311. 1942.

table 1. In the case of McIntosh variety, for instance, a combination was made of infection results with Red Rome on McIntosh, Starking on McIntosh, and own-rooted McIntosh. The combined number of trees in each plot was from 5 to 28, usually about 15. The infection percentages and size of lesions were averaged for 1938, 1939, 1940, and 1941. A high percentage of deep infections will be noted, representing an average of four years' inoculation results. Since the data show a high percentage of positive results, they should have some weight even if they are not based on a large number of individuals. There were some differences in susceptibility (Table 1) as in other experiments on own-rooted varieties previously reported, but unfortunately there was no outstanding resistance in any of the 21 varieties of roots tested.—J. S. Cooley, Senior Pathologist, Division of Fruits and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

Breeding Oats to Combine Resistance to Race 45 and Other Races of Crown Rust Common in Arkansas.—The current, 1944, season marks the first year in which race 45 of crown rust, Puccinia coronata, has been found in such abundance on oats as to constitute a distinct hazard to future oat crops in Arkansas in addition to reducing yields considerably during the current season.

Since 1937, when race 45 was first detected in this State and when Moore, Downie, and Murphy¹ described it, this race has been of minor importance until 1944. It has usually appeared late and seldom in greater abundance than 5 to 10 per cent of leaf area on Bond and its hybrids. Race 45 has been identified by inoculation of the differential hosts used by Murphy. In 1944 it appeared on spring-planted Bond shortly after the boot stage and before anthesis. By the time of the early milk stage fully 30 per cent of the leaf area was infected, and probably more damage was caused than the percentage figure indicates. At that stage most culms had only two green leaves left, the lower leaves having died earlier, apparently as a result of Helminthosporium attacks combined with adverse growing conditions.

On winter oats the effect of race 45 was not nearly so severe because it did not appear in abundance until most of the oats had reached the dough stage. However, its influence on most varieties of winter oats grown at the University Farm, Fayetteville, was not insignificant since the rust was present on Bond hybrids in considerable amounts prior to the maturity of the grain.

Without much doubt, race 45 was also present on most other varieties which possessed no Bond parentage. In general, Victoria hybrids showed about the same reaction to this race as Victoria does to race 1, with perhaps a greater tendency to produce large pustules, although these were still accompanied by some chlorosis and necrosis. The word "perhaps" is used

¹ Moore, M. B., A. R. Downie, and H. C. Murphy. A new race of crown rust that attacks Bond oats. (Abstr.) Phytopath. 28: 16-17. 1938.

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because other race or races were present in abundance, judging by the amount of rust on varieties that are susceptible to one or more races other than race 45. For example, on June 8, Markton was fully 90 per cent infected when Bond averaged about 30 per cent.

Shortly after race 45 was first found in the State, a search was begun for varieties that would be resistant to this race as well as to the races previously found here. One variety, Mutica Ukraina, showed a type of resistance approaching immunity to race 45. Dr. H. C. Murphy² had previously called attention to the high crown rust resistance of this variety, including resistance to races 33 and 34 (published as 34 and 35). Since then, it has been moderately susceptible to a strain of race 1. This strain produces comparatively few infections on this variety and these are characterized mostly by small pustules under natural as well as artificial conditions of infection.

While Mutica Ukraina has little agronomic value, as Dr. Murphy has shown, its potential value for breeding was recognized and it was used extentively by the writer as one of the parents in hybridization work in 1938 and later

Out of this crossing there is now available one hybrid with a high degree of resistance not only to race 45 but to races 1, 7, and several others not so common as these two in Arkansas. It is the only one in a varietal test including 23 varieties and selections which was not severely rusted in 1944.

Parentage of the hybrid is Mutica Ukraina by R3–II. R3–II was derived from a cross made by the writer and L. M. Weetman in the winter of 1936–37, Coker 32–1 × (Victoria × Richland C.I. 3313). The hybrid thus has two different sources of crown rust resistance, Victoria and Mutica Ukraina, and two different sources of smut resistance, Coker 32–1 and Victoria. At present it is known only by its breeding number 674 (2)–39f. Its agronomic characters, which are seemingly quite promising for a spring oat, will be described elsewhere.—H. R. Rosen, Agricultural Experiment Station, University of Arkansas, Fayetteville, Arkansas.

A Penicillium Disease of Soybeans.—During the winter of 1943–1944, numerous lots of soybeans being tested for percentage of germination by the Ohio Seed Improvement Association were examined for the presence of diseases. On many of the plants two types of lesions were common. One type of lesion, found on both cotyledons and hypocotyls, consisted of a sunken, irregular dark brown spot. Another type of lesion was a soft, water-soaked, slightly dark spot on the hypocotyl of germinating seedlings. Similar lesions on the cotyledons of germinating seedlings were unusually large, frequently involving an entire cotyledon which would be a soft light gray mass of decayed tissue.

A species of *Penicillium* was isolated rather consistently from both types of lesions. Cultures of the fungus on both broth and agar were used to inoculate several varieties of soybeans. The results were very striking.

² Murphy, H. C. Making new strains of oats resistant to crown rust by selection and hybridization. Iowa Agr. Expt. Sta. Ann. Rept. 1935-1936: 98-100. 1936.

Inoculated seeds germinated much more slowly than the checks. Those inoculated seedlings which emerged were stunted and the leaves were distorted, being about half the size of normal leaves (Fig. 1). Some stunted

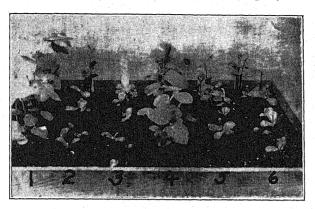


Fig. 1. Symptoms produced by *Penicillium* sp. on soybeans. Rows 1 and 4, checks. Rows 2, 3, 5, and 6, inoculated. Photograph by F. H. Norris.

plants developed lateral buds which resulted in a fasciation effect. The base of the hypocotyl and the cotyledonary leaves of the infected plants had numerous lesions similar to those observed previously on plants in the germinator. On check plants these lesions were almost entirely absent. When cultured, the fungus was readily recovered.

Varieties of soybeans were given a preliminary test of relative susceptibility. Percentages of emergence were as follows:

Variety		Inoculated	d	Checks
Chief		60		100
Dunfield		8		76
Earlyana		36		92
Lincoln		68		96
Manchu		64		96
Mingo		12		96
Richland		56		96
Scioto		28		92

The species of *Penicillium* has not been identified. Studies are under way to determine the percentage of infection this fungus causes under field conditions.—M. R. HARRIS, U. S. Department of Agriculture, Emergency Plant Disease Prevention Survey and C. W. Ellett, Botany Department, The Ohio State University.

Verticillium Wilt and Die-back of Viburnum. —A disease manifested by an unthrifty condition, die-back, and frequently death of Viburnum shrubs was first observed at Lafayette, Indiana, in 1940. Verticillium albo-atrum Rienke and Berth. was isolated from the diseased shrubs, and the capacity of isolates from chrysanthemum, peppermint (Mentha piperita L. var. vulgaris Sole), and Viburnum lantana L. to produce this disease was studied.

¹ Journal Paper Number 174, of the Purdue University Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

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The new growth of infected canes is stunted, the leaves flag, become light green, and then brown when the branches and canes die. Infected canes usually die during the growing season and the brown leaves remain attached until late in the winter. Often only one or a few canes of a shrub may have the symptoms. Sometimes only the branches on one side of a cane are affected. Infected shrubs may succumb in one or two years, or may live in an unthrifty condition for several years. Brown sectors or continuous bands of discolored wood occur and frequently extend from the base to the tip of the infected canes. Only those branches that are contiguous with discolored xylem of a unilaterally infected cane die or display symptoms of the disease. Finally such canes become completely infected and die.

The causal fungus was isolated readily from discolored wood, but not from apparently healthy tissue taken from the distal end of canes.

Verticillium albo-atrum, the causal fungus, was isolated consistently in apparently pure culture from the discolored wood of naturally infected Viburnum lantana and V. tomentosum Thunb. The fungus grew well on potato-dextrose agar and appeared to be typical of V. albo-atrum. Many of the cultures produced variants (saltants), as described for Verticillium by Presley,² Rudolph,³ Thompkins and Ark,⁴ and Tilford and Runnels.⁵ Cultures isolated from Viburnum spp. appeared to be similar to those from chrysanthemum and peppermint in color and in production of pseudo-sclerotia and variant sectors. All cultures formed variants that differed greatly in color, production of pseudo-sclerotia, and conidial sporulation.

Presley² and Rudolph³ showed that the production of pseudo-sclerotia by cultures of V. albo-atrum was extremely variable and that V. dahliae Kleb. in this and other respects did not differ from V. albo-atrum. Tilford and Runnels⁵ although they recognized that the cultures of Verticillium that they isolated from chrysanthemum were unstable, referred them to V. dahliae, since the initial cultures on synthetic medium produced abundant pseudo-sclerotia. The writer concurs with Rudolph³ in considering the production of pseudo-sclerotia an undependable specific character and prefers to refer this species attacking Viburnum to Verticillium albo-atrum.

Shrubs of Viburnum lantana about 24 inches high were planted about the middle of March, 1941, in 12-quart metal pails that had been lined with a coating of tar. Five weeks after planting, four shrubs were inoculated with an isolate from V. lantana, two with an isolate from chrysanthemum, and two with an isolate from peppermint. The inoculations were made by placing one half-pint of a 3-week-old culture of the fungus growing on a mixture of equal parts of corn meal and sand in the soil close to the roots. Four shrubs were treated similarly with sterile medium. The shrubs were

² Presley, John T. Saltants from a monosporic culture of *Verticillium albo-atrum*. Phytopath. 31:1135-1139. 1941.

Rudolph, B. A. Verticillium hydromycosis. Hilgardia 5: 197-353. 1931.
 Thompkins, C. M. and P. A. Ark. Verticillium wilt of strawflower. Phytopath.
 1130-1134. 1941.

⁵ Tilford, Paul E. and Harmon A. Runnels. Verticillium wilt of chrysanthemums and its control. Ohio Agric. Exp. Stat. Bull. 630. 1942.

grown in a greenhouse during the spring of 1941, then were placed outdoors during the summer and returned to the greenhouse in October. In May, 1942, the surviving shrubs were placed outdoors and remained there until the next year. The buckets, when outdoors, were buried in the ground to within two inches of the top, and were kept well watered.

One plant that had been inoculated with the isolate from *V. lantana* showed symptoms of "Verticillium hydromycosis" 8 months and the remaining three plants 13 months after inoculation. The two shrubs that were inoculated with the isolate from chrysanthemum developed typical symptoms 6 and 9 months after inoculation. One of the shrubs inoculated with the isolate from peppermint developed typical symptoms of "Verticillium hydromycosis" 25 months after inoculation and died 4 months later. The other shrub inoculated with this isolate died from an undetermined cause a few months after inoculation. The four control shrubs remained healthy during the test. The fungus was reisolated from all of the infected shrubs and appeared to be similar to the original cultures.

The results of inoculating Viburnum lantana with the three isolates of Verticillium albo-atrum indicated that possibly the isolate from peppermint was less pathogenic than the isolates from chrysanthemum and V. lantana. The three isolates were tested on chrysanthemum, variety Amoskeag; eggplant, variety N. H. Hybrid; pepper, variety California Wonder; and pimiento, variety Sweet Meat Glory, to determine if they differ in pathogenicity to these hosts. Four-week-old cultures of the isolates growing on a mixture of equal parts of corn meal and sand were incorporated in flats-of-soil that had been steam-sterilized. Eight eggplant, pepper, and pimiento plants, about one month old and between $2\frac{1}{2}$ and $3\frac{1}{2}$ inches high, and 8 small chrysanthemum plants were planted in the infested soil in April and allowed to grow until July. A like number of the plants also were planted for "controls" in soil in which similar, but sterile, medium was incorporated.

The eggplants in the soil infested with the isolates from chrysanthemum and Viburnum lantana were stunted and approximately half of the plants were partially defoliated and the remainder defoliated, except for the terminal leaves, 6 weeks after transplanting. The eggplants in the soil that was infested with the isolate from peppermint showed symptoms of "Verticillium hydromycosis" but were not stunted nor defoliated as severely as with the other two isolates. Cultures of Verticillium albo-atrum that were reisolated from a number of the infected eggplants appeared to be similar to the original isolates. The inoculated chrysanthemum, pepper, and pimiento plants remained uninfected. Control plants of the eggplant were not infected.

The results, although not conclusive, are suggestive of variation in pathogenicity in the cultures of V. albo-atrum studied. This is similar to the results of Tilford and Runnels who reported "forms" of Verticillium differing in pathogenicity on chrysanthemum.—R. C. Baines, California Department of Agriculture, Sacramento, California.

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NOEL FINLEY THOMPSON 1891–1944

JAMES G. DICKSON

Noel Finley Thompson, Associate Plant Pathologist, Wisconsin Department of Agriculture, died April 27, 1944. He had known for years that leukemia would terminate his work yet in his characteristic tranquil and confident manner he gave full service to the State and prepared for the final day. Natural teacher, inquisitive investigator, and honest administrator, he elevated regulatory work to cooperation in insect and disease control. His passing is a pronounced loss to phytopathology, the State of Wisconsin, and his wide circle of associates and friends.

Mr. Thompson was born at Oakland, California. The family later moved to Yakima, Washington, where he completed high school in 1910. He attended Whitworth College three years, transferred to the University of Washington, and completed the requirements for the B.S. degree in 1915. He held a teaching fellowship in botany at the University and the Puget Sound Biological Station in 1916 and 1917 and completed the requirements for the M. S. degree in Botany in 1916. He served six months in the armed forces in 1918. Upon release he was appointed Assistant Pathologist in the Office of Cereal Investigations, United States Department of Agriculture, on wheat smut eradication in the Pacific Coast area. In 1919 he accepted a summer appointment as instructor and Acting Head of the Botany Department at his Alma Mater and served as Assistant Professor of Botany at the University of Idaho the following year. He entered the graduate school, University of Wisconsin, in the autumn of 1920 and simultaneously assumed the responsibility for the barberry eradication program in the State. His characteristic devotion to his job soon resulted in a full-time appointment in barberry eradication, first in the Bureau of Plant Industry and later in the Bureau of Economic Entomology and Plant Quarantine, United States Department of Agriculture. In 1927 he joined the staff of the Wisconsin State Department of Agriculture and in this capacity served well the nurserymen, florists, eranberry growers, and other plant culturists until the time of his death.

On December 23, 1922, he married Grace Vivian Bitterman of Madison, Wisconsin. His wife and three children, Noel James, in the armed forces, Ellen Grace, and Elizabeth Anne, survive. Mr. Thompson was a member of the American Phytopathological Society, the Wisconsin State Horticultural Society, the Wisconsin State Gladiolus Society, the Wisconsin Academy of Science, Sigma Xi, Gamma Alpha, and Phi Sigma.

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NOEL FINLEY THOMPSON 1891-1944

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USTILAGO STRIAEFORMIS. III. A FURTHER STUDY OF FACTORS THAT INFLUENCE AFTER-RIPENING OF CHLAMYDOSPORES FROM POA PRATENSIS¹

K. W. KREITLOW 2

(Accepted for publication September 18, 1944)

INTRODUCTION

In an earlier paper (15) on chlamydospores of Ustilago striaeformis (West.) Niessl in smutted leaves of Poa pratensis L., it was shown that treatment in a moist chamber at 32° to 35° C. reduced the after-ripening period of the spores from 197 days to less than 30.

Although races of Ustilago striaeformis with germinable chlamydospores have been found (2, 6, 14), in the vast majority of collections tested, only a negligible percentage of the spores germinated. Davis (3) succeeded in germinating spores of the smut only after they were exposed to a prolonged after-ripening period. Similar after-ripening periods of varying duration have been described for chlamydospores of other smuts. Since Noble (19) reviewed much of the literature on factors influencing germination of smut spores, only the more recent work will be discussed.

REVIEW OF LITERATURE

Many workers have found that freshly gathered spores of some smuts fail to germinate as readily as spores kept for varying periods at laboratory temperature (13, 17, 24). Holton (10), working with a race of Tilletia tritici, failed by several different treatments to induce germination of the typical, dark chlamydospores, while hyaline spores among the dark chlamydospores were capable of immediate germination. In contrast, Rump (23) found that dark chlamydospores of Ustilago hordei germinated readily while light brown or hyaline spores failed to germinate.

Several investigators have studied the stimulatory effect of light, gases, chemicals, presence of plant tissues, and temperature on germination of chlamydospores of different smuts. Light stimulated germination of spores of Tilletia tritici (9, 22), but if the spores were suspended in a medium that contained certain nitrogenous salts, light was not necessary. Ling (16) found that spores of Urocystis occulta germinated better in darkness or diffuse light.

The presence of oxygen was necessary for germination of spores of Ustilago avenae (12), U. zeae (21) and U. hordei (25). Concentrations of carbon dioxide up to 15 per cent also had a stimulatory effect (20). Davis (3) found that spores of U. striaeformis exposed to fumes of chloroform and

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¹ Contribution No. 66, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the northeastern states.

then submerged in a citric acid solution germinated earlier than untreated spores. A similar response was secured when spores of several smuts were exposed to ether vapors (5).

Chemical agents such as salts of light metals in weak concentrations and nitrogenous salts stimulated germination of chlamydospores of *Tilletia tritici* (9, 22). Spores of *Urocystis tritici* (19) and *U. occulta* (16, 26) germinated readily after treatment in a weak solution of benzaldehyde.

Crushed or uninjured plant tissues of different kinds as well as germinating seeds have a stimulatory effect on germination of chlamydospores of *Urocustis tritici* (8, 18, 19), *Ustilago avenae* (4), and *U. zeae* (20).

Spores of several species of *Urocystis* were induced to germinate after soaking in water at low temperatures for varying periods (7, 11). Kreitlow (15) shortened by several months the after-ripening period of spores of *Ustilago striaeformis* from *Poa pratensis* by incubating detached smutted leaves at 35° C. in a moist chamber. Since effective study of *U. striaeformis* is dependent on securing an abundant supply of germinable chlamydospores, further efforts were made to determine the nature of the response of chlamydospores to the incubation treatment.

MATERIALS AND METHODS

Chlamydospores were collected from two smutted clones of *Poa pratensis* propagated from single tillers and maintained in a greenhouse. The composite sample used in some of the tests was composed of the pooled smutted leaves of five different clones of Kentucky bluegrass. The chlamydospores were obtained by scraping spores from mature sori with a flattened needle or by macerating smutted leaves in water in a Waring Blendor (1).

Germination of chlamydospores was tested in quadruplicate by suspending spores in drops of distilled water on microscope slides (14). Each slide was placed in a Petri-dish moist chamber and the chlamydospores were incubated 16–20 hours at 25° C.

Methods for testing the factors considered likely to hasten after-ripening of spores are discussed under each heading. The factors studied were: Contaminating organisms, host tissue, abrasion or scarification of chlamydospores, temperature, and moisture.

EXPERIMENTAL RESULTS

Influence of Contaminating Organisms on the After-ripening of Smut Chlamydospores.—Fresh, smutted leaves of Poa pratensis were cut into lengths of approximately one inch. The leaves were rinsed in a 1:500 solution of mercury bichloride in 50 per cent ethyl alcohol for 15 seconds and then thoroughly washed in several changes of sterile water. Following this treatment, the leaves were placed in sterilized moist chambers and incubated at 35° C. At periodic intervals, pieces of leaves were removed aseptically from the moist chambers and the spores were scraped from smut sori for a germination test.



Germinable chlamydospores were obtained from the surface-sterilized leaves 10 to 25 days after incubation was started. In some of the tests, the spores required a longer period to attain maximum germinability. In some cases germination failed to exceed 30 per cent, but this may have been due to a residual effect of mercury bichloride. The results indicated that contaminating organisms did not shorten the after-ripening period of the smut chlamydospores.

Influence of Host Tissue on the After-ripening of Smut Chlamydospores.—Fresh, smutted leaves of Poa pratensis were cut into 1-inch lengths, placed in several hundred cc. of distilled water and macerated for one minute in a Waring Blendor. Following this treatment, residual plant material was removed by straining the spore suspension through a piece of cheesecloth. Chlamydospores suspended in the plant juice and water were concentrated by centrifuging the suspension at low-speed for several minutes in an angle centrifuge. Most of the supernatant liquid was decanted and the spores were resuspended in the remaining liquid and collected by pouring the suspension on a fine grade filter paper.³ That portion of the paper retaining the greatest concentration of filtered chlamydospores was cut into small bits and placed in a Petri-dish moist chamber for incubation at 35° C. At the same time a sample of the spores was tested for germination.

Another suspension of chlamydospores in plant juices and water was subjected to low-speed centrifugation and the spores were resuspended in sterile water. The cycle of washing and centrifuging chlamydospores was repeated three times. After each washing, some of the spores were resuspended in 10 cc. of sterile water and collected on filter paper for incubation at 35° C. Samples of spores for immediate germination tests were collected at each stage of the operation.

None of the chlamydospores removed at different stages of treatment for an immediate test proved germinable. Tests at frequent intervals on spores incubated at 35° C. in moist chambers revealed that spores washed several times were more germinable than unwashed spores or those washed only once. A germination of 35 to 50 per cent was obtained after incubation for 12 days with the spores washed three times compared with 25 per cent for spores washed once and 10 per cent for unwashed spores. Presence of host tissue, however, was not necessary for hastening after-ripening.

Abrasion as a Factor Hastening After-ripening of Smut Chlamydospores.—During the course of experiments to free chlamydospores of host tissue, it was observed that the after-ripening period for spores treated in the Waring Blendor frequently was reduced by several days. It seemed possible that the treatment may have scarified the spores and thus hastened germination.

Fresh, 10-gram samples of smutted leaves of *Poa pratensis* were placed in 400 cc. of distilled water and treated in a Waring Blendor for 15 seconds, 30 seconds, 60 seconds, 3 minutes, and 5 minutes. A control was prepared

3 Schleicher and Schüll Co., Inc., New York, N. Y. No. 589, 12½ cm.

by scraping spores from leaves of the same plants into 50 cc. of distilled water. After each interval of treatment, a 50-cc. sample of the spore suspension was removed for concentrating and washing in the angle centrifuge. Each spore sample was washed three times and the spores collected on filter paper for incubation at 35° C. Germination of the treated spores was tested every other day.

Chlamydospores from two different clones and from a composite sample of five different clones of Kentucky bluegrass all responded similarly. Spores from the control required 10 to 15 days to after-ripen at 35° C. Most of the spores agitated in the blender after-ripened 3 to 10 days following treatment and incubation at 35° C.

There was no definite increase in germinability of chlamydospores with length of treatment time. Spores agitated 15 seconds seemingly germinated as rapidly and as well as spores treated five minutes. Conversely, there was no apparent injury from the longer treatment periods.

Agitating spores in the blender not only hastened germination but also gave a definite increase in germination over untreated spores. Whereas rarely more than 75 per cent of untreated spores germinated, often 90 per cent of treated spores germinated.

Influence of Temperature on the After-ripening of Chlamydospores Agitated in a Blender.—Chlamydospores were removed from fresh, smutted leaves of each of two clones of Poa pratensis by macerating the leaves in a Waring Blendor for one minute. The spores from each clone were washed three times in an angle centrifuge and collected on filter paper. Pieces of filter paper bearing spores from the separate clones were placed in Petri-dish moist chambers for incubation at 5°, 25°, and 35° C. Spores from each set of dishes were removed for an immediate germination test. Germinability of the spores was determined thereafter at 5-day intervals.

Tests with chlamydospores incubated at each of the three temperatures showed that after-ripening was hastened only in those spores stored at 35° C. Of chlamydospores stored at 5° and 25° C., less than 10 per cent germinated during a 30-day storage period, while those stored at 35° C. attained a maximum germinability, for this experiment, of 75 per cent within 15 days.

Effect of Storage at Lower Temperatures on Subsequent After-ripening of Chlamydospores.—Chlamydospores from each of the two clones of Poa pratensis used in the previous experiment were maintained in moist chambers at 5° and 25° C. for 60 days. The Petri-dish moist chambers containing the spores were then transferred to an incubator at 35° C. for after-ripening of the chlamydospores.

When spores were stored at 5° C. an after-ripening period of 10 to 20 days at 35° C. was necessary to secure maximum germination, while chlamy-dospores maintained at 25° C. before they were transferred to 35° C. required only 3 to 5 days to reach maximum germinability. This suggested that partial after-ripening may have occurred in the spores stored at 25° C.

Moisture as a Factor Influencing After-ripening of Smut Chlamydospores.—Chlamydospores were obtained from fresh, green, smutted leaves the re ty on an

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of a single clone of *Poa pratensis* (Clone 37KB120(26)) by macerating the leaves in a Waring Blendor for varying lengths of time. The spores were washed three times, collected on filter paper, and placed, with a control, at different moisture levels in an incubator at 35° C. Spores stored dry were placed inside Petri dishes containing dry sheets of filter paper. Spores incubated partially dry were placed inside small, dry, open dishes which in turn were placed inside a Petri-dish moist chamber. This permitted the filter paper bits containing the spores to remain dry while the spores themselves were maintained in a humid atmosphere. Chlamydospores incubated wet were placed inside small covered dishes containing wet filter paper and these

TABLE 1.—Germination of chlamydospores agitated for varying lengths of time in a Waring Blendor and incubated for after-ripening at 35° C. under different moisture conditions

Agita-	Moisture		Percenta	ge germi	nation 0 t	o 31 days	s after in	cubation	
time	MOISTAIG	0	2	5	7	9	12	19	31
Check	Dry Partly dry Wet	few	few 0-few few-5	few-5 few 15	few-10 0-few 50	0-few 0 90	few 0 90	few 5 50–75	few 10-25 10-25
15 sec.	Dry Partly dry Wet	few-5	5 few few-5	few-5 few 50-75	few-5 few 90	few 0 75–90	few-5 0-few 90	few 0-few 90	few 10-25 50-90
30 sec.	Dry Partly dry Wet	5–10	few-5 5-10 10	5–10 few–5 75	few-5 0-few 30-50	few 0-few 90	5 0 90	few few 90	few 25-50 90
60 sec.	Dry Partly dry Wet	few-5	few-5 few few	5 few 25–50	few-5 0-few 75-90	5 0-few 75-90	few 0 75–90	5 0-few 75	few 25 10–25
3 min.	Dry Partly dry Wet	few-5	5 few-10 5	few-5 few 75-90	5 0-few 75-90	few 0-few 75-90	0-few 0-few 90	few 0-few 50	few 10-25 25-50
5 min.	Dry Partly dry Wet	few	few few-10 15-30	few 5–10 75	0-few few-5 90	0-few 0-few 90	0-few 10-50 90	0-few 25-75 90	0 10–75 75

dishes in turn were placed inside Petri-dish moist chambers. In this manner, the spores on the filter paper bits were in direct contact with moisture and were insured against drying-out at the high temperature. The dishes in each condition were examined daily and the water replenished when necessary. Germination was tested for spores in each environment at frequent intervals.

As shown in table 1, after-ripening of smut chlamydospores occurred when the spores were maintained wet at 35° C. Some after-ripening apparently occurred among spores stored under partially dry conditions since germinability had increased to 25 to 50 per cent after 31 days. Also, spores agitated five minutes began to after-ripen under partially moist conditions 10 to 15 days earlier than spores treated for shorter periods or not treated at all. Chlamydospores stored dry at 35° C. failed to after-ripen regardless of treatment.

DISCUSSION

Treatments have been found that reduce the after-ripening period of the chlamydospores of stripe smut on *Poa pratensis* from 197 days to less than 30 days. The prolonged soaking used by Davis was replaced by incubating chlamydospores at 35° C. in a moist chamber. Since many factors probably are involved in after-ripening, an effort was made to determine which were primarily responsible.

The influence of contaminating organisms was eliminated when spores within surface-sterilized host tissues were made germinable in a reasonable time by proper incubation treatment. Presence of host tissue was found unnecessary since spores washed free of plant juices and after-ripened on filter paper were germinable after a short incubation period. While freeing spores from host tissue by macerating smutted leaves in a Waring Blendor, it was observed that spores so treated frequently germinated more abundantly and earlier than untreated spores, possibly because of abrasion or scarification of the spores. Chlamydospores treated in the blender, however, still required incubation at 35° C. in a moist chamber before they became germinable.

Partial after-ripening may occur when spores are stored at certain temperatures. Spores stored moist at 25° C. for three months after-ripened in 3 to 5 days when incubated at 35° C. In contrast, spores stored at 5° C. required 10 to 20 days' incubation.

The importance of adequate moisture was demonstrated when spores incubated dry at 35° C. failed to after-ripen while spores incubated in contact with free water reached maximum germinability in a short time. Chlamydospores incubated in a saturated atmosphere but not in direct contact with water after-ripened only partially. It was observed that few spores germinated during the incubation treatment at 35° C. despite intimate contact of spores and water. Germination occurred only after treated spores were placed in drops of water and incubated at a lower temperature for 16 to 20 hours.

SHMMARY

Chlamydospores of *Ustilago striaeformis* from *Poa pratensis* were afterripened in host tissue free of contaminating organisms. Spores washed free of host tissue were after-ripened on filter paper bits incubated at 35° C. in a moist chamber.

Treatment of chlamydospores in a Waring Blendor hastened after-ripening and enhanced germinability.

, Fresh chlamydospores stored at 5° C. for 60 days and then transferred to 35° C. required longer to after-ripen than a similar set of spores stored at 25° C.

Water in contact with the spores was necessary for successful after-ripening. Spores incubated in a saturated atmosphere required longer incubation, while spores incubated dry failed to germinate.

UNITED STATES REGIONAL PASTURE RESEARCH LABORATORY, STATE COLLEGE, PENNSYLVANIA. he re ty \mathbf{on}

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SYNTHETIC CULTURE MEDIA FOR THE ROOT-ROT FUNGUS, PHYMATOTRICHUM OMNIVORUM¹

WALTER N. EZEKIEL2

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In studies (6) on nutritive relations of *Phymatotrichum omnivorum* (Shear) Duggar, a synthetic culture solution designated as "solution 70" was derived by successive modification of Brown's artificial potato-dextrose medium (2). Later, solution 70 proved a convenient basic medium for testing additions of either nutrient or possibly inhibitory materials (1, 5, 13). Talley and Blank (13) found that this solution was well balanced and that growth of the fungus was not improved significantly by increasing or decreasing any of the major inorganic components. Growth increased with additional nitrogen (as ammonium nitrate) and carbon (as dextrose) but efficiency of carbon utilization decreased.

Several workers (1, 7, 10, 11) proved that substrata for *Phymatotrichum omnivorum* were improved by additions of zinc, iron, manganese, and sometimes copper. In extensive experiments, Blank (1) found that with unpurified solutions, greater growth was obtained with additions of salts of zinc, iron, and manganese to provide about 2 ppm. of these metals. With solutions purified with calcium carbonate, copper was beneficial also, but additions of copper to unpurified solutions were sometimes detrimental.

The results summarized in table 1 illustrate the relative importance of these metals in the nutrition of *Phymatotrichum omnivorum*. Traces of heavy metals were first removed from solution 70 by the Steinberg (12) technique (autoclaving with calcium carbonate and filtering) and salts of the metals then added. Growth was initiated from agar discs cut from plate cultures of *P. omnivorum*, isolate 57, grown on 135-agar (6). In agreement

TABLE 1.—Growth of Phymatotrichum omnivorum in purified solution 70 with the designated additions of heavy metals. (Mean values of quintuplicate cultures in 25 ml. portions of substrata, incubated at 28.5° C.)

Composition of nutrient solutions, indicated as		Mean dry weight of colonies, after			
additions to purified solution 70	3 weeks	4 weeks	5 weeks		
	mg.	mg.	mg.		
No additions(lacks Zn, Fe, Mn, Cu)	35	35	54		
Plus 2.5 ppm. Fe, Mn, Cu (lacks Zn)		54	67		
do Zn, Mn, Cu (lacks Fe)		141	192		
do Zn, Fe, Cu(lacks Mn)	232	287	307		
do Zn, Fe, Mn (lacks Cu)	251	314	333		
do Zn, Fe, Mn, Cu; complete ^a		336	338		

a This complete solution is designated subsequently as solution B.

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² Formerly Pathologist, Texas Agricultural Experiment Station; now Senior Mycologist, Bureau of Ordnance, Navy Department.

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with the results of Blank (1), lack of zinc reduced growth most sharply, lack of iron somewhat less, and lack of manganese or copper to a still lesser extent. (By contrast, additions of gallium to the complete solution had little effect on growth of the fungus.)

In recent work, the various synthetic media have generally been used with additions of the metals. For convenient reference, and to avoid the continuing circumlocution of repeatedly describing these media as deviations from the old formulas, the more important formulas as now used are assembled below.

In preparing these media, additions of the small amounts of heavy metals are conveniently made from stock solutions prepared so that 1-ml. portions will furnish 2.5 ppm. for 1 liter of final culture solution. For 100-ml. volumes of the stock solutions, use respectively: $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.10 g.; $\text{Fe}_2(\text{SO}_4)_3$, 0.90 g.; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.77 g.; and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.98 g.

Solution A is essentially the old solution 70 with Zn, Fe, and Mn added. The basic solution contains per liter:

MgSO ₄ ·7H ₂ O	0.75	g.
K ₂ HPO ₄	1.35	76
KCl		"
Ammonium nitrate	1.18	"
Dextrose	40.0	"

Zn, Fe, and Mn sulphates are added in amounts to furnish 2.5 ppm. of each metal.

Solution B is the same as solution A, except that the basic solution is purified (by autoclaving with 15 g. of calcium carbonate per liter for 20 minutes at 15 lb. and filtering while hot) and addition then made of Cu as well as Zn, Fe, and Mn, each at 2.5 ppm. This solution has been used, for example, in testing possible growth-promoting materials (4) and in measuring the inhibitive effect of various compounds (8, 9).

Solution C, modified from the old solution 81 (6), provides a more highly buffered solution than A or B, and has been used in study of pH ranges of isolates of the fungus. It contains per liter:

$MgSO_4 \cdot 7H_2O$	1.0	g.
K ₂ HPO ₄	2.0	71
Asparagin	2.0	"
Peptone	2.0	. 6 6
Dextrose	40.0	"

plus 2.5 ppm. each of Zn, Fe, and Mn.

Agar D is based on 135-agar (6), which was useful as a general culture medium for *Phymatotrichum omnivorum* and some other fungi. For stock cultures and in various experiments (3), agar D has proved better with *P. omnivorum* than potato-dextrose agar. This medium contains per liter:

MgSO ₄ ·7H ₂ O	0.75 g.
K ₂ HPO ₄	1.5 "
Peptone	4.2 "
Ammonium nitrate	1.2 "
Sucrose	

plus 2.5 ppm. each of Zn, Fe, and Mn, plus agar 20 g.

Agar E. Copious development of sclerotial masses around walls of flask cultures has been obtained with 134-agar (6), which differed from 135-agar by inclusion of 40 g. per liter of commercial corn starch. With the further addition of the heavy metals, this is now designated as agar E. Make up as agar D, but incorporate 40 g. of starch before adding the agar. (The starch is conveniently first stirred into about 100 ml. of cold water, mixed next with about 400 ml. of boiling water, then with the other ingredients which have been dissolved in the remainder of the water, and the agar finally added.)

AGRICULTURAL EXPERIMENT STATION,

COLLEGE STATION, TEXAS.

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ROOT DISEASE OF CASTANEA SPECIES AND SOME CONIFER-OUS AND BROADLEAF NURSERY STOCKS, CAUSED BY PHYTOPHTHORA CINNAMOMI

BOWEN S. CRANDALL, 1 G. F. GRAVATT, 2 AND MARGARET MILBURN RYAN2

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INTRODUCTION

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Soon after extensive work on chestnut blight was started in 1912, reports of a disease of chestnut were received from regions where it seemed unlikely that blight had yet gained a foothold. Dying chestnuts, Castanea dentata (Marsh.) Borkh., were reported at the lower elevations in the southern Appalachians from Virginia to Georgia, and in the mountainous and hilly regions of west Tennessee, north Alabama, and north Mississippi. Observations by various individuals disclosed that the dying was caused by a root disease. It was first supposed that Armillaria mellea Fr. was responsible, since it was the only pathogenic organism isolated from the rotted tissue. In 1931, using new techniques, a Phytophthora was isolated by the writers from the roots of dying American chestnut and chinkapin trees and was eventually identified as P. cinnamomi Rands and so reported by Tucker (33). The same fungus was causing a destructive root rot of forest-tree nursery stock. This paper reports the work leading to the identification of the root-rotting Phytophthora as P. cinnamomi and the inoculation and field tests that proved its pathogenicity on chestnut and other hosts.

HISTORY OF THE PARASITE

For over 100 years chestnut trees (Castanea sativa Mill.) in Europe have been dying from a root rot called the "ink disease." It was first reported from Portugal in 1838 and elsewhere in Europe soon after, but no detailed studies prior to those of Gibelli (15), about 1879, are known. Numerous other pathologists, especially French and Italian, tried to discover the cause of the disease, which they attributed, among other things, to Armillaria mellea, Coryneum perniciosum Briosi and Farneti, mycorrhizal fungi that had become parasitic, bacterial complications, and poor soil conditions (12). Finally, about 1917, Petri isolated the true causal organism and classified it as Blepharospora cambivora Petri (26), later changed to Phytophthora cambivora (Petri) Buis. Later P. cinnamomi Rands also was reported associated with the "ink disease" of chestnut and beech (Fagus sylvatica L.) in England (11) and chestnut trees in southern Europe (22).

Associate Pathologist, Office of Foreign Agricultural Relations; formerly Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Athens, Ga., in cooperation with the School of Forestry, University of Georgia, Athens, Ga.

² Respectively, Senior Pathologist, and formerly Assistant Scientific Aide, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Plant Industry Station, Beltsville, Maryland.

In the United States the record of dying Castanea can be carried back to 1824 with some certainty. In that year chinkapins were reported dying from unknown causes in Georgia (18). The general recession of the chestnut trees in the Southern States has been reviewed by Clinton (4). Reports of old residents described the dying of chestnut and chinkapin trees at lower elevations in north Mississippi, Alabama, Georgia, Tennessee, Virginia, Maryland, and the Carolinas. Around 1900 the Ozark chinkapin (Castanea ozarkensis Ashe) was dying in Arkansas, Missouri, and Oklahoma except on the tops of high, dry ridges in the Ozarks.

In 1930 Phytophthora cinnamomi was first reported in the United States by White (37) on rhododendron. No Phytophthora was reported on Castanea in this country until 1932 when Milburn and Gravatt (24) isolated a Phytophthora from dying C. dentata and C. pumila (L.) Mill. This Phytophthora later was identified as P. cinnamomi Rands. In 1933 P. cinnamomi was found to be the cause of a destructive root rot of red pine (Pinus resinosa Ait.) in nursery seedbeds (16). A number of coniferous and broadleaf seedlings and transplants later were found to be subject to root rot in the nursery or in plantings (6). Before the discovery that P. cinnamomi was responsible, the losses were attributed by some nurserymen to high water table and by others to heavy soils. These conditions favor attack by Phytophthora. According to the files of the Division of Mycology and Disease Survey, Beltsville, Md., P. cinnamomi was reported by Plakidas in 1939 on tung in Louisiana and by Wager in 1940 on avocado in southern California, In 1938 P. cambivora was first reported in the United States, on maple (Acer platanoides L.) in New Jersey (29). The authors never have found it associated with root rot of chestnut or nursery stock in the United States.

In 1936 Mehrlich (23) questioned the validity of separating *Phytophthora cinnamomi* Rands from *P. cambivora* and suggested that "they be combined into a single species *P. cambivora* in which strain differences may be recognized." In 1937 White (38) placed the rhododendron wilt fungus in the combined species *P. cambivora*. In 1936 the name *P. cambivora* (*P. cinnamomi*) was used in reporting root rot of nursery stock caused by *P. cinnamomi* (6). Some confusion has resulted from this combination of species. The two species, as separated and recognized by European authorities and by Tucker in this country, are present in the United States. The writers are keeping the two species separate.

SYMPTOMS

Above-ground symptoms of this root disease vary considerably. In some cases in early summer, typical wilting of the leaves appears, followed by death and defoliation of the tree. In other, more typical cases the slow loss of roots is first indicated by a gradual reduction in the size of the leaves over a period of several years (Fig. 1, B). This condition usually is accompanied by chlorosis and slight wilting. In some such cases the entire tree dies during the dormant season. In other cases the branches die back until

only a few are left; these may remain alive for several years (Fig. 1, C and D).

The symptoms of the disease on and in the roots are similar to those

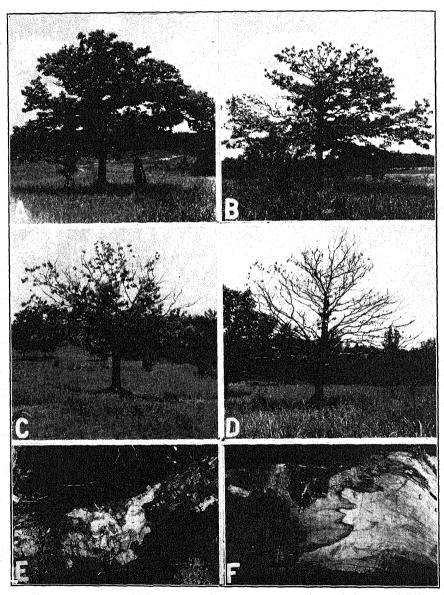
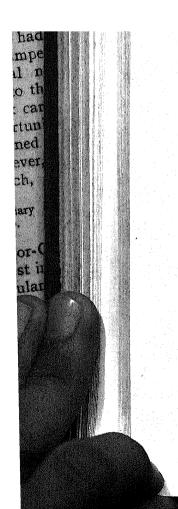


Fig. 1. A. Healthy-appearing chestnut tree. B. Early symptoms of root rot. C and D. Advanced symptoms of root rot. E. Surface symptoms of root-rot lesion. F. Dissection, showing advancing wedge-shaped streaks.

- described in Europe for the "ink disease." The lesions on the roots usually may be detected by the presence of an inky-blue exudate that stains the soil close to the root. The surface of a lesion is brownish-black (Fig. 1, E).



The lesions vary from small round spots to areas extending a number of feet along a root. Eventually, the lesions coalesce and girdle the root, and the tree dies when the collar is girdled or when most of the roots are killed. Often the first lesions are on the taproot well below the root collar.

When a lesion is dissected, recently invaded tissue is commonly mottled light brown and green with no definite margin. The fungus generally advances from the invaded region into sound tissue in irregular, wedge-shaped streaks (Fig. 1, F). While essentially a root parasite, the causal *Phytophthora* can often be found above the ground line in the collar region.

EPIDEMIOLOGY

Recession of the American Chestnut

Early in the study of the chestnut root rot it became evident that the facts being brought to light probably accounted for the steady recession of the chestnut from large areas in the Gulf and Atlantic States to the foothills and mountains of Mississippi, Alabama, Georgia, Tennessee, Maryland, Virginia, and the Carolinas. Buttrick (3), in a report based on field work done in 1912–1913, states, "During the last seventy-five years the range of chestnut in North Carolina has decreased considerably. It was formerly found throughout the western Piedmont section, and remains of old stands and single isolated trees are still to be met with. Along the eastern slopes of the Blue Ridge and occasionally at the lower elevations in the mountains themselves, the recession may still be observed going on, trees are in poor health and dying off and their places are being taken by other species. The cause of this recession is not well understood, though various reasons have been advanced."

We are accustomed to thinking of introduced epidemic diseases as something more or less peculiar to this century, characterized as it is by speedy means of communication. The history of *Phytophthora cinnamomi* as we have been able to reconstruct it indicates that the fungus probably came to the United States more than a hundred years ago, possibly by way of trading ships operating between one of our southern ports and the East Indies or Asia. The importation of numerous exotic plants for the gardens of antebellum estates could easily have been the direct means of its entry. Once introduced, it apparently spread slowly westward, northward, and inland, its hosts being the American chestnut and chinkapins. Some observers noted the dying of northern red oaks (*Quercus borealis* Michx. f.) where this species occurred mixed with dying chestnut and chinkapins. This species, although highly susceptible in nurseries, has not been proved a host for *P. cinnamomi* in forest stands.

In the course of the survey work on the disease, numerous reports were obtained from old residents giving the story of the death of the chestnuts and chinkapins, either as they remembered it or as it was told to them.³ An

³ A resident from McCutchins Cove in the Cumberland Mountains of Tennessee stated that he remembered seeing the floor of the valley covered with a heavy stand of chestnut. All chestnuts suddenly sickened and died within a year or two, except a few along the

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or-\ st ii interesting characteristic of the root disease, noted by many of the early observers and by the writers, is the simultaneous decline and death of large numbers of trees in one locality. Often almost all of the trees of these species in areas of many acres or in an entire valley died within 2 or 3 years. Isolated trees within these areas remained healthy-appearing for years. The dates of the disappearance of the chestnut and chinkapins as given in these reports and in literature are plotted on the map in figure 2. Shaded areas show the regions where the writers noted symptoms of the root disease. The ranges of the American chestnut and chinkapins are shown, and the locations where *Phytophthora cinnamomi* has been isolated are marked.

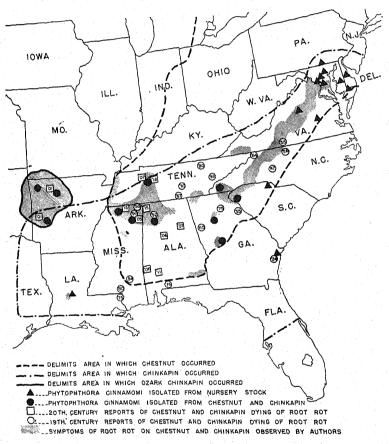


Fig. 2. Observed range of root rot caused by Phytophthora cinnamomi.

Recession of the Ozark Chinkapin

About 1900 a duplication on a limited scale of what probably happened to the chestnut occurred through the range of the Ozark chinkapin. Reports

tops of the hills. He placed the date accurately as 1867, since it was the year when soldiers were returning from military prisons in the North, and the residents of the Cove thought that the disease was something carried from the Yankee prisons by the returning soldiers.

of residents and Forest Service personnel then stationed in the area indicate that most of the chinkapin population on bottom land and at low elevations died within a few years. The root rot is active on survivors of the original epidemic and the species is slowly disappearing. Only a few large mature trees remain, at high elevations in northwest Arkansas.

Local Spread

How the *Phytophthora* originally reaches an area is not known, but various means of transfer are possible. As soon as *Phytophthora* appears in an area, the trees growing on the lowest, poorest-drained sites are the first to die. Those on heavy soils are affected first. The fungus then spreads from tree to tree from the lower areas to the higher dryer areas. Trees on loose, sandy, well-drained sites frequently remain unaffected for years. On moist sites with heavy soils there is some evidence that the fungus infects the roots at numerous places after spreading through the soil from the original infection court.

ISOLATION FROM CASTANEA SPECIES

Late in 1931 specimens of roots from dead and dying American chestnut trees were sent to the Division of Forest Pathology from an orchard in Georgia. Cultures from the affected areas yielded a fungus identified as a *Phytophthora* and similar to *P. cambivora*, cause of the "ink disease" in Europe. However, as certain differences were noted, comparisons of this chestnut root rot fungus with *P. cambivora* and *P. cinnamomi* were made. This same *Phytophthora*, subsequently determined to be *P. cinnamomi*, was isolated from American chestnut specimens from other localities in Georgia and Tennessee, from *Castanea pumila* in South Carolina, and from *C. crenata* Sieb. and Zucc. in Louisiana. In 1935, in a survey made through the ranges of the various species of *Castanea* in the Southern States, *P. cinnamomi* was isolated from American chestnuts dying of root rot in Mississippi and Alabama, from Ozark chinkapin in Arkansas, and from the Alabama chinkapin (*C. alabamensis* Ashe) in Alabama.

The *Phytophthora* appeared in only 4 per cent of the cultures from naturally infected specimens shipped in by mail. In the field, where isolations were made as soon as the roots were dug, the percentage was only slightly higher, but quite variable. The most important factor in the successful isolation of *Phytophthora* is to locate healthy tissue that is just being actively invaded. Under such conditions the pathogen is growing ahead of other fungi that overwhelm it in culture. The best times of the year for isolating the pathogen from diseased roots appear to be spring and fall; however, isolations have been made in every month of the year. Day (11) reported that in England *P. cinnamomi* was isolated from *C. sativa* only during September and November. Difficulty in isolating *Phytophthora* from walnut (31) and chestnut (13) has been attributed to the presence of toxic exudates as well as saprophytic organisms. In the present study, especially where

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the specimens were shipped in by mail, molds and particularly Trichoderma were fruiting on the surface of the bark. Trichoderma has been found fruiting on Phytophthora lesions on roots at the time they were dug. Since Trichoderma has been reported parasitic on various soil fungi, including a species of Phytophthora (36), possibly it may be parasitic on the Phytophthora on chestnut roots. In tests, 9 of 12 isolates of Trichoderma found as contaminants in cultures where the chestnut Phytophthora was expected, apparently were able to parasitize P. cinnamomi in pure culture. However, nursery field tests in which the soil was inoculated with Trichoderma in an attempt to control Phytophthora gave negative results.

The best method for isolating *Phytophthora cinnamomi* was to place sections of diseased root tissue containing only the advancing edge of the rot and about one-fourth inch of healthy tissue in test tubes containing sterile water. Sections from chestnut should be kept in water for 2 or 3 days, several changes of water being desirable. The sections then should be lightly surface-sterilized and plated on corn-meal or prune agar. Typical growth of *P. cinnamomi* can often be detected in a few days.

A method suggested by Dr. C. M. Tucker, inserting rot sections in apple and subsequently isolating *Phytophthora* from the apple tissue, has been especially good for infected native species of *Castanea*. Sections of rot tissue are selected and surface-sterilized, and the outer bark is removed. Small slivers of wood and inner bark containing infected and healthy tissue are cut and forced into an apple through a cut in the skin. The hole should be sealed with tape or vaseline. This method fails at times because of contamination by the vigorously growing apple parasite, *Diplodia natalensis* P. Evans.

THE PATHOGEN

Description

Better growth was obtained on oatmeal agar made by Tucker's formula (32) than on any other medium employed; on it the fungus produced an abundant aerial growth of tangled, white hyphae and chlamydospore-like bodies. Similar growth occurred on sterilized carrot slants. On corn-meal agar there was little aerial growth and many of the hyphal tips were swollen and variously shaped (Fig. 3, C). The same kind of round bodies appeared on lima-bean agar.

The scant growth that appeared on steamed corn meal was tangled, white, and aerial, like that on the oatmeal agar. Medium-length aerial hyphae with vesicles in the submerged growth occurred on carrot agar. In nutrient solution made according to Petri's formula (27), some vesicles were produced; however, they were more abundant in sterile tap water cultures (Fig. 3, B). The hyphae were hyaline and averaged 5.2 μ in width, with a range of from 4.7 to 6.2 μ . They had only occasional cross walls and contained many fat globules. The chlamydospore-like bodies (Fig. 3, A) were round and thick-walled, averaged about 29 μ in diameter, and usually appeared singly. The vesicles (Fig. 3, B) were irregular in shape and size,

were thin-walled, and occurred in bunches. Petri believed that vesicles act as conservation agents since the extraordinary abundance of vesicles in the Correze form of *Phytophthora cambivora* corresponded to its almost constant sterility.

Sporangia and zoospores were produced only when the *Phytophthora* was grown in running water. The cultures were started on carrot slants or oatmeal agar and then transferred to running water. A piece of the agar culture was cut from the dish and tied in cheesecloth before it was put in water. Even with this method the appearance of sporangia was erratic; sometimes they developed quickly and at others not at all.

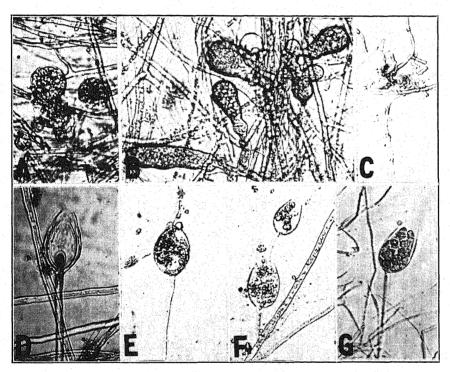


Fig. 3. Phytophthora cinnamoni Rands from chestnut: A. Chlamydospore-like bodies. B. Production of vesicles in sterile tap water. C. Typical hyphae with swollen tips growing on corn-meal agar. D. New sporangium forming inside emptied sporangium. E. Hypha growing from apex of sporangium. F. New sporangium produced on sporangiophore arising from base of old sporangium. G. Sporangium and zoospores.

Slightly papillate sporangia without distinct plugs were produced at the ends of long, unbranched sporangiophores and averaged 55.0 \times 38.8 μ . Other reported sporangia sizes for *Phytophthora cinnamomi* have been 57 \times 33 μ (30), 43.6 \times 25.9 μ , and 46.1 \times 31.3 μ (32). *P. cambivora* has been reported as having sporangia 47.6 \times 31.1 μ (32), 37.4 \times 23.7 μ (21), and 60–75 \times 40–54 μ (27). The contents of the sporangium generally differentiated into zoospores (Fig. 3, G). Just before the mouth opened to liberate them there was considerable motion inside the sporangium; then an opening appeared

at the apex, and the zoospores squeezed out. They swam around vigorously for a time before coming to rest, and initiated growth by producing a single unbranched hypha. In some cases the contents of the sporangium escaped without first becoming differentiated and seemed simply to disintegrate. At other times the sporangium germinated without spore production, a hypha growing out from the apex (Fig. 3, E).

After a sporangium emptied, another usually formed inside the old case (Fig. 3, D); sometimes there were as many as four old cases outside a young developing sporangium. Often, a sporangiophore grew out of the base of an emptied sporangium and through the mouth, elongated, and gave rise to a new sporangium (Fig. 3, F). Very rarely a sporangiophore was observed growing out from immediately below an evacuated sporangium.

Since a number of persons who have worked with *Phytophthora spp.*. among whom are Leonian (20), McRae (22), and Narasimhan (25), believe them to be heterothallic, isolates from various localities in Georgia, Tennessee, and South Carolina were grown together on the chance that they might represent different segregates. Seven of them, paired in all possible combinations, were grown on oatmeal, corn-meal, potato-dextrose, and malt agars, and in Petri's solution, tap water, and distilled water, but no oogonia resulted. Furthermore, no oogonia were found under any conditions under which the isolates were studied.

Comparison with Phytophthora cambivora

The presence of both *Phytophthora cinnamomi* and *P. cambivora* in the United States makes a comparison between these species of interest. At the time of the study *P. cambivora* had not yet been found in this country and, therefore, no isolates of this species from the United States were included in the tests. Three isolates of *P. cambivora* from chestnut in Europe were compared with a number of isolates made by the writers and with 8 of *P. cinnamomi* from the following sources: 3 of Rands' from cinnamon in the East Indies, 1 of Petri's from chestnut in Europe, 1 of Cookson's from walnut in Australia, 1 of Sideris' from pineapple in Hawaii, 1 of Mehrlich's from Hawaii, 1 of White's from rhododendron. Each isolate was not used in every comparison.

No differences between isolates of the chestnut P. cinnamomi, greater than any of those between the other isolates, were noted; therefore, all isolates of P. cinnamomi are considered as a group.

Isolates of *Phytophthora cinnamomi* and *P. cambivora* were pathogenic to apple and nonpathogenic to eggplant. Those of *P. cinnamomi* were pathogenic to potato and green tomato, while those of *P. cambivora* were nonpathogenic. These results are in accord with Tucker's findings (32) on the same hosts.

No significant macroscopic differences were apparent when the isolates of *Phytophthora cinnamomi* and *P. cambivora* were grown on oatmeal, cornmeal, or malt agars. Growth on malt agar was very irregular for all strains.

On all these media, the isolates of *Phytophthora cinnamomi* were characterized microscopically by the presence of irregularly swollen hyphal tips and grape-like bunches of chlamydospores or chlamydospore-like bodies. No such growth was present in the *P. cambivora* isolates. Dark chlamydospores were present in cultures of *P. cinnamomi* on KNO₃ media; none were present in cultures of *P. cambivora* on the same media.

Optimum temperatures for growth were about 25° C. for *Phytophthora cinnamomi* and about 30° C. for *P. cambivora*. White (38) reported 25–27.5° C. for *P. cinnamomi*, while Tucker (32) reported 25–27° C. for *P. cinnamomi* and 27–30° C. for *P. cambivora*.

Leonian (19) found considerable difference in reaction to the presence of malachite green in various concentrations in nutrient solution. He reported sporadic growth of $Phytophthora\ cambivora\$ at $\frac{1}{16}$ and $\frac{1}{8}$ ppm. of dye and no growth at $\frac{1}{4}$ ppm., whereas $P.\ cinnamomi\$ grew sporadically at $\frac{1}{4}$ ppm. of dye. In our tests isolates of $P.\ cambivora\$ made some growth at $\frac{1}{16}$ ppm. of dye and none in stronger solutions, whereas isolates of $P.\ cinnamomi\$ showed slight growth in concentrations up to and including $\frac{1}{2}$ ppm. of dye.

No oogonia were ever found in the writers' isolates of *Phytophthora cinnamomi*, although cultures were grown in comparison with other isolates of *P. cinnamomi* and *P. cambivora* on media suggested by previous workers: water agar (1), synthetic agar (1), KNO₃ (32), soil agar (5), grated-carrot agar (17), and carrot-extract agar (28). Oogonia have been reported in old cultures of *P. cinnamomi* by Ashby (2) and on KNO₃ by Tucker (32). Ashby (2) has reported them in old cultures of *P. cambivora*, while Petri (28) found them in this species on carrot-extract agar.

INOCULATIONS

Potted plants in the greenhouse were inoculated; the majority with aerial hyphae produced on oatmeal agar. Wound inoculations were generally made on the main root, a short distance below the collar. In tests without wounds, the inoculum, agar cultures with as little agar attached as possible, was planted in the soil. Results obtained from this type of inoculation indicate that wounding did not increase the percentage of infection.

When infested soil was the inoculum source, the plants were removed from soil in which they were growing and replanted in soil in which other inoculated plants had previously died. The *Phytophthora* was able to live at least 1 or 2 months saprophytically in the soil and to kill plants transplanted into it.

Representatives of 5 tree genera were inoculated. The oaks and oak-like *Lithocarpus* were included because of their close relationship to *Castanea*, and walnut and beech because *P. cambivora* had been reported on them (9, 10).

As shown in table 1 the European and American species of Castanea were much more susceptible than the Oriental species to isolates of the chestnut Phytophthora cinnamomi. The percentage figures for susceptibility are in

most cases the average of the results of inoculations with several different isolates of P. cinnamomi from chestnut. Some of the isolates killed 100 per cent of the American species of Castanea while others were comparatively weak. Comparisons between isolates are further discussed under the nursery phase of the root-rot problem. It is interesting that in Europe C. mollissima and C. crenata have also been reported as resistant to P. cambivora (14). In

TABLE 1.—Susceptibility of various tree species to isolates of Phytophthora cinnamomi from chestnut and chinkapin

Species	Plants inocu- lated	Killed by Phytoph- thora	Check plants	Dead ^a
	No.	Per cent	No.	Per cent
Castanea alabamensis Ashe	140	70	30	57
C. alnifolia Nutt.	113	82	30	57
C. ashei Sudw.	16	94	4	0
C. crenata Sieb, and Zucc.	227	4	101	1
C. dentata (Marsh.) Borkh.	86	70	54	2
C. henryi (Skan) Rehd. and Wils.	81	2	. 50	0
C. margaretta var. arcuata Ashe	10	60	2	0
C. mollissima Blume	197	1	102	0
C. ozarkensis Ashe	60	70	12	25
C. pumila (L.) Mill.	107	74	30	23
C. sativa Mill,	251	38	124	2
C. seguinii Dode	8	0	4	0
Fagus grandifolia Ehrh.	31	0	24	0
F. sylvatica L.	40	0	5	0
Juglans nigra L.	59	0р	36	0
Lithocarpus cuspidatus (Thunb.) Nakai	3	0	3	0
L. densiflorus (H. and A.) Rehd.	6	50	8	0
Quercus alba L.	75	3	57	0
Q. agrifolia Née	10	0	5	0
Q. borealis Michx. f.	56	4	28	0
Q. coccinea Muench,	13	. 0	13	0
Q. garryana Dougl.	54	0	30	0
Q. macrocarpa Michx.	18	0	12	0
Q. marilandica Muench.	21	0	10	0
Q. montana Willd.c	157	1	80	0
Q. palustris Muench.	69	0	37	0
Q. phellos L.	25	0	12	0
Q. prinus L.	13	0	11	0
Q. rubra L.	38	0	37	0
Q. velutina Lam.	29	0	15	0

a Phytophthora could not be isolated from any of the dead check plants.

a laboratory test C. dentata was susceptible to one of the European isolates of P. cambivora.

The 3 species of Quercus that were infected artificially with Phytophthora cinnamomi were later found naturally infected in nurseries (6). Neither American beech (Fagus grandifolia) nor European beech (F. sylvatica) became infected. This is an interesting difference between P. cinnamomi and P. cambivora, since in England (11) P. cambivora has been found causing root rot on European beech, and P. cinnamomi has not been reported from this host.

b In later experiments isolates from J. regia L. and J. nigra L. were able to kill 40 per cent of 5-year-old trees, while a virulent strain from red pine caused no root rot. c Called Q. prinus (24).

Black walnut was not infected, under the conditions of the experiment, with any of the isolates of *P. cinnamomi* from chestnut. Later these same trees were successfully inoculated with isolates from root rot of seedling black walnuts (*Juglans nigra* L.) and Persian walnuts (*J. regia* L.).

Some years ago, Dr. N. I. Vavilov, of the Institute of Plant Industry, Leningrad, U.S.S.R., visited the chestnut breeding and disease plots of the Bureau of Plant Industry near Washington and was considerably interested in the possible susceptibility of the Russian chestnuts, which grow so extensively in the Caucasian Mountains, to this root disease and to the chestnut blight. Arrangements were made for an exchange of seed, whereby Dr. Vavilov sent seed to the United States in 1933 from the Russian chestnut trees and the Division of Forest Pathology sent him seed of some of its more promising hybrids to test against Russian diseases.

Inoculations with *Phytophthora cinnamomi* showed that the Russian chestnuts were fully as susceptible to the root disease as were the other strains of the European chestnut that this Division tested. The Russian chestnuts from the Caucasian Mountains were also moderately susceptible to the chestnut blight disease, caused by *Endothia parasitica*. No information has been received from Dr. Vavilov for some time about the reactions of our chestnuts to the Russian diseases.

FIELD TESTS OF ASIATIC CHESTNUTS

Test plantings of Asiatic chestnuts have been established on soils in areas where the root disease is active on native Castanea or where it has appeared on nursery stock or in orchards. These plantings were in Alabama, Arkansas, Georgia, Maryland, Mississippi, Louisiana, and Tennessee. From these tests it has been determined that all available strains or selections of Castanea crenata, C. mollissima, C. henryi (Skan) Rehd. and Wils., and C. seguinii Dode have a high degree of resistance to Phytophthora cinnamomi, which causes only small localized lesions on the roots. Individuals of C. sativa, known to be susceptible to root rot, were placed in the plantings to act as checks. These trees died within a year or two. Resistant trees remained in the plots from 5 to 11 years and were unaffected by the root disease. In two isolated cases where seedlings of Castanea crenata and C. mollissima were growing in waterlogged soils, losses from root rot have occurred, but how much damage resulted from water and how much from fungus was undecided.

PHYTOPHTHORA ROOT ROT OF NURSERY STOCK

Hosts

In 1933, following early spring floods, heavy losses were experienced in beds of 3-year-old red pine (*Pinus resinosa*) in a Maryland nursery. The disease was first thought to be of physiological origin because of the failure to isolate a pathogen by any recognized technique. However, the disease continued to spread notwithstanding the return of more or less normal conditions in the nursery. With the water-blank method described previously

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a *Phytophthora*, later found to be *P. cinnamomi*, was isolated and its pathogenicity was proved (16).

For several years losses, often of epidemic proportions on the more susceptible species,4 occurred in seedbeds and plantings of various tree species in the nursery where this disease was first noticed and in other nurseries as follows: Pinus resinosa from 2 nurseries in Maryland and 1 in Delaware; P. sylvestris L. from 1 nursery each in Maryland and Virginia; P. strobus L. from 1 Maryland nursery; Taxus cuspidata Sieb. and Zucc.* from 3 nurseries in Maryland and 1 in Virginia; T. baccata L.* and T. media Rehd.* from 1 nursery in Maryland; Picea abies Mill. from 1 nursery each in Maryland and Virginia; P. pungens Engelm. from 1 nursery in Maryland; Larix decidua Mill.* and L. leptolepis (Sieb. and Zucc.) Gord.* from 1 Maryland nursery; Juglans nigra* from 2 Maryland nurseries and 1 each in North Carolina, Arkansas, and Louisiana; J. regia* from 1 Maryland nursery; Quercus borealis* and Q. montana from 2 Maryland nurseries; and the following each from 1 nursery in Maryland: Rhododendron mucronulatum Turez.,* Betula alba L., B. papyrifera Marsh.,* Platanus orientalis L., Robinia pseudoacacia L., and Quercus alba.

Symptoms

On both coniferous and broadleaf species the fungus commonly causes a root rot of seedlings and transplants. While apparently uncommon, an instance is known in a single nursery where *Phytophthora cinnamomi* initially produced typical damping-off. On seedlings, the above-ground symptoms of the typical root rot are first noticed in the late spring or summer. The first indication of trouble is the loss of the normal green of the foliage, followed by wilting in the broadleaf species and by dieback and less obvious wilting in the coniferous species. On older nursery stock the symptoms in the above-ground portions usually do not appear until the root system is almost completely rotted. The tree, therefore, appears to decline rapidly, with death soon following. Leaf symptoms on such trees often appear after the causal organism has become inactive and are not uncommonly attributed to other causes, such as drouth or twig blight.

On coniferous stock beyond the damping-off age, the fungus causes a dry type of root rot accompanied by resin deposition on and in the infected portions, including the wood. This infiltration of the wood at the point of attack is particularly valuable as a distinctive symptom, since fungi that kill bark and cause external resin flow usually do not cause resin deposition in the xylem. The areas invaded by the fungus are reddish-brown. The very recently invaded tissue is characteristically mottled brown and light green, except in species of *Taxus* where recently infected tissue is lightly streaked with black.

On broadleaf species the rot produced is dry, except in the case of *Robinia* and *Juglans*. Members of these two genera also differ in that the color of ⁴ Indicated by asterisk.

the rot is not typical. The rot produced on *Robinia* is the same color as the healthy surrounding tissue, while on *Juglans* the rot is black. In all other species seen, the rot is reddish-brown, and, like that on the conifers, is mottled brown and light green in recently invaded tissue.

In both coniferous and broadleaf species no definite line of demarcation is found between the invaded and healthy tissue. The fungus advances into sound tissue in irregular wedge-shaped streaks. Phytophthora cinnamomi apparently is capable of direct penetration into healthy cells and was not found in cultures made far behind the zone of active penetration. Data taken from an inoculation experiment on ninety 3-year-old Pinus resinosa give an indication of its rate of spread within the host. Thirty-two days after inoculation 61 per cent of the population was dead. The average spread of the fungus recorded at the time girdling and death of these trees occurred was 9 cm. up and 5 cm. down from the point of inoculation, or 2.8 mm. up and 1.5 mm. down per day. The fastest spread of the fungus during this period was 14 cm. up and 5 cm. down from the inoculation point, or 4.3 mm. up and 1.5 mm. down per day.

Phytophthora cinnamomi apparently often attacks just below the soil line and frequently occupies a portion of the stem of infected trees. The presence of the fungus in tissue above the soil line is often indicated, in conifers, by external resin flow, and, in broadleaves, by a depressed area above the dead cambium.

In the United States, except in the case of Juglans nigra, no exactly similar diseases of the hosts, caused by other fungi, have been described. A disease of walnut identical in symptoms but caused by another member of the same genus, Phytophthora cactorum, has been reported (8). It can be distinguished from P. cinnamomi root rot only by cultural methods. As yet, the fungi have not been found together on walnut, although both have been found in the same nursery. They have, however, been found together in the same seedling in cases of late damping-off of red pine.

A root disease of *Pinus resinosa*, differing somewhat in symptoms, has been found in the same nursery and occasionally on the same tree as the Phytophthora root rot. The symptoms of this disease, caused by *Sphaeropsis ellisii* Ell. and Ev. (7), differ from those produced by the *Phytophthora* in that the infected tissue is deeper red with black streaks running through it and into the xylem. No resin deposition is associated with this rot.

Inoculations

Inoculation tests with *Phytophthora cinnamomi*, using four to fifteen 2- to 5-year-old vigorously growing potted trees, with re-isolation of the pathogen, agree with the field observations that *Pinus resinosa*, *Larix decidua*, and *Juglans nigra* are highly susceptible. *J. nigra* was susceptible to isolates from *J. nigra* and *J. regia* in Maryland, but not susceptible to isolates from *P. resinosa* in Maryland or *Castanea dentata* in Georgia. It was not possible to apply Koch's rules of proof to a number of the individual

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ity lor lar 7 t. ass l f species from which P. cinnamomi was isolated, because they were not available for inoculation. Twenty isolates from 16 different hosts growing in seven states have, however, all proved to be identical in that they can produce the root disease on P. resinosa and C. sativa and can be reisolated from such cases of rot. Species such as Picea abies and Robinia pseudoacacia, found only slightly susceptible in the field, were completely resistant in the inoculation tests. As a result of the greenhouse inoculations Douglas-fir (Pseudotsuga taxifolia (Poir.) Britton) has been found highly susceptible to root rot caused by isolates from C. pumila in South Carolina, J. nigra and P. resinosa in Maryland, and Taxus cuspidata in Virginia. Fagus grandifolia and F. sylvatica were not susceptible to isolates from Maryland, Delaware, Virginia, and Georgia. P. mugo var. mughus (Scop.) Zenari, tested because it is grown as an ornamental associated with yews and often is used to replace yews that die of root rot, was not susceptible to any of the isolates. No root rot has ever been found on this species in nurseries or plantings. The results of the cross inoculations indicate that there are probably a number of slightly different physiologic strains of P. cinnamomi. In a small laboratory test, one of Rands' original isolates (30) of P. cinnamomi from Cinnamomum burmanni Nees caused root rot of Pinus resinosa that was indistinguishable from that caused by isolates from that host. The isolate was, however, only weakly parasitic, as compared with isolates from red pine, since it took about 4 times as long to invade an equivalent amount of tissue.

Conditions Predisposing to Attack

As previously mentioned, the first observed loss on *Pinus resinosa* was an epidemic apparently induced by abnormally wet soil conditions. Subsequent losses of this and other tree species have been sporadic. Generally such losses have been heavy. In the instances where a period of abnormally wet weather was not the apparent predisposing factor, the trees involved usually had been planted on a poorly drained site. While conditions of poor soil aeration create a favorable condition for attack by the fungus, when a susceptible host is invaded the fungus apparently is capable of killing it regardless of soil or weather conditions.

An experiment was made to determine the effects of soil and water on the incidence of the disease, with 3-year-old seedlings of *Pinus resinosa* taken from beds in which the disease was present. The trees selected apparently were free from root rot, but no attempt was made to wash or sterilize the roots. Thirty trees were potted in greenhouse soil and an additional 30 in a mixture of half soil and half sand. One third of each group were given only enough water to keep them alive, another third 3 times as much, while the remaining third were watered 6 times as much. At the end of 118 days, in the groups given the largest amount of water, 8 seedlings in soil and 4 in the soil-sand mixture were dead; of the group given 3 times the minimum watering, 4 in the soil and 2 in the soil-sand mixture were dead; while of those given the minimum amount of water, 3 in the soil and 2 in the soil-sand

mixture were dead. Phytophthora cinnamomi and in some of the trees Sphaeropsis ellisii as well were isolated from the dead trees. Wager (35) was able to artificially infect avocados with P. cinnamomi when pots in which they were growing were submerged in water for periods as short as 2 or 3 days. Infection was not possible with trees given normal watering.

Phytophthora cinnamomi has been isolated from root-diseased nursery stock growing in soils ranging in pH from 3.2 to 7.0. In a single experiment, in which each of 20 pots, with soil adjusted to pH values of 4.2, 5.0, 7.0, and 8.0, were planted with 1 inoculated and 1 healthy Pinus resinosa tree, results were inconclusive since an equal number of the healthy trees under each pH value contracted the disease. The finding of the fungus causing root rot on nursery stock growing in soils with a considerable pH range would seem to indicate that while the fungus may be affected by an adverse pH, as has been shown by White (38), it is nonetheless able to grow and infect susceptible hosts. Once infected, the effect of pH probably would be more important as it affected the vigor of the host itself, rather than the fungus. Field tests to control root rot in seedbeds of red pine and blue spruce by adjusting the acidity with sulphur and aluminum sulphate were unsuccessful. The fungus continued its activities at pH readings as low as 3.3, at which point the host was injured by the acid condition.

CONTROL

No satisfactory control measures for this disease in the nursery have been developed. When rot is observed on stock being transplanted, all infected individuals should be isolated, and preferably destroyed. At least they should not be planted near healthy susceptible species. In nursery soil known to be infested with *Phytophthora cinnamomi*, poorly drained sites and sites on which losses have occurred should be avoided or adequate draining should be provided when planting susceptible species. Care should be taken to avoid overdense stands in the seedbeds of susceptible species.

All sanitation practices that tend to stop movement of infested soil and plant material within the nursery should be used. Such soil and plant material should never be added to a compost heap. For such species as Juglans nigra, which can be planted directly in the forest, seedbed planting in areas where losses from Phytophthora cinnamomi have occurred should be held to a minimum.

Before work on this disease was started, blight had already appeared on American chestnuts in the areas where Phytophthora root rot was found. Absence of any effective control for blight, in either orchard or forest chestnuts, made control of root rot immaterial. Asiatic chestnuts, brought to the United States because of their blight-resistant qualities, are also resistant to root rot. Hybrid varieties, resistant to both blight and root rot, are being developed by crossing American chestnut and chinkapins with Asiatic species. In Spain, Urquijo (34) reports control of root rot, presumably caused by *P. cambivora*, by removing the soil from the collar region of infected trees, cleaning the affected area after the fungus is presumably killed by

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desiccation, applying a sticker, and then dusting with copper carbonate. This protection is reported to last for a number of years after the soil is refilled around the collar region. The majority of root-diseased chestnuts examined in the southeastern United States usually had most of the main lateral roots infected as well as the collar region. Often death occurred because most of these lateral roots were girdled without the collar region becoming involved. However, it appears that the control described might be practical if the host tree involved was less susceptible than the American chestnut. Varieties of the European chestnut are in orchard plantings in the United States, and our tests show them to be less susceptible than the American chestnut to *P. cinnamomi*. In blight-free areas top working of root-rot susceptible varieties on resistant Asiatic root stock offers an obvious control method.

SUMMARY

Phytophthora cinnamomi Rands has been found causing root rot, similar to the "ink disease" of chestnut in Europe, on the American chestnut in Maryland, Virginia, North and South Carolina, Georgia, Alabama, Tennessee, and Mississippi. It has been found causing the same disease on Castanea ozarkensis in Arkansas. Other native chinkapins have been killed by the Phytophthora where they occurred within the range of the chestnut. It seems probable that this disease is responsible for the recession of the American chestnut from the lower Piedmont area of the Atlantic States into the higher elevations of the mountains and from southern Mississippi and Alabama into the hilly regions in the northern parts of those States. It is known to have caused the recession of the Ozark chinkapin. The pathogenicity of P. cinnamomi has been demonstrated on its native chestnut and chinkapin hosts.

In addition, this fungus is the cause of a similar root disease on 20 coniferous and broadleaf hosts growing in nurseries in Arkansas, Delaware, Georgia, Louisiana, North Carolina, Maryland, and Virginia.

Field and greenhouse inoculation tests have demonstrated that the European chestnut, Castanea sativa, is susceptible to the disease, while the Asiatic species, C. crenata, C. mollissima, C. henryi, and C. seguinii, have a high degree of resistance to it.

In greenhouse inoculation tests, Douglas-fir (*Pseudotsuga taxifolia*) was highly susceptible to the root rot *Phytophthora*.

Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration,

U. S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, Md., IN COOPERATION WITH SCHOOL OF FORESTRY, UNIVERSITY OF GEORGIA, ATHENS, GEORGIA.

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BLIGHT OF ORIENTAL ARBORVITAE

A. G. PLAKIDAS

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Several varieties of the oriental arborvitae (Thuja orientalis L.) are extensively grown in Louisiana and other parts of the South as ornamental plants. For several years¹ these have been subject to a very destructive disease known locally as ''blight'' or ''fire.'' The two varieties most commonly grown in Louisiana, Berckman's Golden and Baker, are very susceptible. Another variety, the Rosedale, which was once very popular, is virtually out of existence at present because of this disease. The same trouble, apparently, affects also the Italian cypress (Cupressus sempervirens L.). The symptoms on this host are similar to those on arborvitae, and the fungus proven experimentally to be the cause of the blight on arborvitae appears, morphologically and culturally, identical with that on diseased Italian cypress. However, no cross-inoculation tests have been made.

The exact geographical distribution of the disease is not known. It is definitely known that, besides Louisiana, it is present in the neighboring states of Texas, Mississippi, and Arkansas, and it is presumed to occur in other parts of the South where arborvitae are grown.

In the past the disease has been variously attributed to red spider, drought, excessive summer heat, or winter injury. A critical study during the past two years has shown definitely that the disease is caused by a fungus, an apparently undescribed species of *Cercospora* (6). Because of the importance of the disease, and because preliminary experiments have shown that it is amenable to control with copper sprays, the results of the investigation are presented at this time with the hope that they may be of interest to other investigators and of practical use to nurserymen and others. Work is in progress to determine the host range of the fungus, the period during which infection takes place, and the most practical method of control.

DESCRIPTION OF THE DISEASE

Affected leaves and branchlets are killed, turn brown, and fall off gradually. The shedding of the branchlets leaves the plant thin and ragged (Figs. 1 and 2, A). Approximately the lower two-thirds of the plant is the most severely affected. Usually there is a tuft of healthy growth at the top of even severely diseased plants. Very often, one side of the plant will be practically killed with only scattered dead areas on the other side. This is particularly true of plants growing close to houses: the side away from the wall is usually more severely diseased than the one next to the wall. The disease is more severe in nurseries than in gardens, probably because the plants in the nursery are close together and the fungus spreads easily from

¹ No authentic records regarding the earliest appearance of the disease have been found, but from talks with nurserymen and others, it was learned that blight has been known probably for 20 to 25 years.

plant to plant. In the nursery row, infected plants are often killed in a relatively short time (1-3 years); in gardens, affected plants may persist in a ragged, unhealthy condition almost indefinitely.

Cankers also have been observed on young stems. These girdle the bark and kill the small twigs. The portion of the stem immediately above the ringed bark swells considerably (Fig. 2, B) giving the appearance of an insect gall. Stem cankers, thus far, have been found only on one variety,

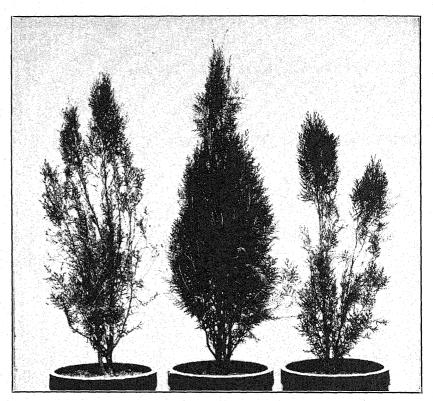


Fig. 1. Contrast between check (center) and inoculated arborvitae, variety Baker. The plants were inoculated with pure cultures of *Cercospora thujina* July 14, 1943, and photographed May 20, 1944. The illustration shows the thin, ragged condition of the diseased plants which results from the shedding of the diseased branchlets, but does not show the brown color of the dead foliage. ×0.1.

Berckman's Golden. The same fungus that causes the leaf blight has been isolated from the stem cankers.

CAUSE OF THE DISEASE

Isolations. A fungus, apparently a Cercospora, has been constantly associated with the disease. The fungus was first isolated from diseased material of the Baker variety collected in a nursery in Folsom, Louisiana, April 6, 1943. Since then it has been found on specimens of diseased arborvitae collected from different parts of Louisiana and on one specimen re-

ceived from Arkansas. Cultures of the fungus have been obtained both by planting pieces of diseased leaves on agar and by isolating single conidia.

In addition to the *Cercospora*, perithecia of *Mycosphaerella sp.* were often found on diseased material, either alone or in combination with *Cercospora*. The *Mycosphaerella* was obtained in culture by isolating single ascospores. As isolates of the two fungi appeared similar in culture, it was at first assumed that the *Mycosphaerella* represented the ascigerous stage of

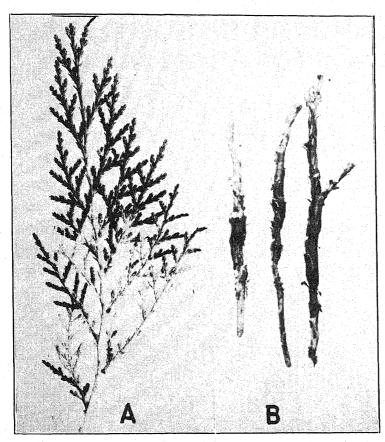


Fig. 2. A. Arborvitae (var. Baker) twig showing difference in color between healthy and diseased foliage and branchlets. B. Bark cankers on stems of Berckman's Golden variety. Both approx. natural size.

Cercospora. However, the Mycosphaerella proved non-pathogenic to arborvitae (Table 1), and it must be assumed that it has no connection with the Cercospora.

Pathogenicity. All the inoculations were made on healthy arborvitae, variety Baker, about 3 feet in size and planted in 4-gallon crocks. As the fungi used in these experiments did not sporulate in culture,² the inoculum

² Of a total of 29 isolates of *Mycosphaerella*, two produced a few conidia in culture. These varied from one-celled elliptical to 2-septate fusiform-cylindrical. None of the 54 isolates of *Cercospora* sporulated in culture.

was prepared by macerating the mycelium, together with the agar substratum, in water by means of a mechanical blender according to the method suggested by Andrus (1). Young cultures, 9–12 days old, grown on potato-dextrose agar, were used to make the inoculum, which was sprayed on the plants by means of a small insect sprayer. The inoculated plants were placed under large bell jars for periods ranging from 48 to 96 hours, then removed and placed outdoors. Inoculations were made also by placing diseased, conidia-bearing twigs on healthy plants.

The results of the inoculation experiments are in table 1. Infection with *Cercospora* was obtained in every case. On plants inoculated during the summer (July 14 to September 20) infection was heavy, approximately 50 to 75 per cent of the foliage becoming necrotic by the end of 5 to 6 weeks after inoculation. The period lapsing between inoculation and the appear-

TABLE 1.—Summary of inoculation experiments on Thuja orientalis L., var. Baker, with Cercospora thujina and Mycosphaerella sp.

Treatment	Time of treatment	No. plants		
reatment	Time of treatment.	Inoculated	Diseased	
Cercospora thujina Pure culture Conidia-bearing twigs	Summer Winter July 14 and Aug. 4, 1943	8 4 3	8 4 3	
Mycosphaerella sp. Pure culture	Summer	7	0	
Check Not treated Atomized with H_2O , placed in moist	Summer	71	0	
chamber	Summer Winter	4 3	0	

^a Summer inoculations were made on July 14, Aug. 4 and 14, Sept. 2, 9, and 20, 1943; winter inoculations were made on Jan. 6, Feb. 8, and Mar. 17, 1944.

ance of disease symptoms varied considerably in the different experiments. In the first inoculation made on July 14, the early symptoms (yellowish discoloration of the leaves) appeared in 2 weeks, and necrotic lesions in 4 weeks after inoculation. In all other inoculations made during the summer, first evidence of infection was apparent about 4 weeks after inoculation and typical necrotic lesions in 5 to 6 weeks.

With inoculations made during the winter (January 6, February 8, and March 17), an entirely different situation was encountered. The inoculated plants were examined once a week until April 25, and, except for a trace of disease on one small twig of one of the two plants inoculated on January 6, they remained apparently healthy. It was concluded then that the results of the winter inoculations were negative and that infection apparently did not occur during the cool season. However, when the plants were reexamined on May 15, all were typically diseased, although the infection was much lighter than on the plants inoculated during the summer. It is not

known whether infection occurred at the time of inoculation and the disease did not develop during the cool season, or whether the fungus remained viable externally on the leaves and produced infection with the advent of warm weather. This question is important from the control point of view. Development and spread of the fungus in natural infections have not been noted during the winter, although viable conidia have been found on diseased plants during the winter months.

The symptoms produced by inoculation were typical of the disease as it occurs naturally. The fungus sporulated abundantly on the inoculated plants. It was reisolated many times, and it produced the disease when reinoculated into healthy plants.

The Mycosphaerella sp. (29 isolates) was used to inoculate 7 plants on 6 different dates (Table 1), and in not a single case did it produce infection. It must be concluded, therefore, that it has no connection either with the disease or with the Cercospora which is the cause of the disease.

THE FUNGUS

Identity

No report of Cercospora on Thuja has been found in literature, although this genus has been reported on related conifers. Ellis and Everhart (2) reported C. Sequoiae on Sequoia gigantea, and C. Sequoiae var. Juniperi on Juniperus virginiana. Satisfactory specimens of the type material of these were not seen by the writer. A small fragment of Ellis and Everhart's type specimen of C. Sequoiae on Sequoia gigantea was obtained from the Farlow Herbarium through the kindness of Dr. D. H. Linder, but neither sporophores nor conidia were found on it.

Three specimens (2 herbarium and 1 fresh) of Cercospora Sequoiae var. Juniperi, all on Juniperus communis var. depressa, were examined. One, obtained from the Farlow Herbarium, was collected by J. J. Davis in Melvina, Wisconsin, June 12, 1916. The second was obtained from Dr. Charles Chupp, of Cornell University, and was collected by H. C. Green in Madison, Wisconsin, June 10, 1943. The third, which was fresh, was received directly from H. C. Green and was collected in the same locality on August 16, 1943. Since the original description of C. Sequoiae var. Juniperi by Ellis and Everhart was based on the specimen collected by J. J. Davis in Wisconsin, it is reasonably safe to assume that Davis' determination of the fungus on J. communis var. depressa was accurate, and, therefore, that the Wisconsin specimens examined by the writer actually represented C. Sequoiae var. Juniperi E. and E.

The same fungus was found on all three specimens. It was studied rather critically in comparison with the fungus occurring on arborvitae, and it was concluded that the two are entirely distinct. The juniper fungus does not appear to be a *Cercospora*, and, in fact, it appears doubtful that it belongs even in the same family with *Cercospora*. Its fruiting body (Fig. 3, D) is composed of a thick sclerotial stroma which extends deeply into the

parenchyma tissue and becomes erumpent, forming a raised, cushion-like structure on the surface of the leaf from which arise very numerous short conidiophores. The fruiting body is definitely a sporodochium; the fungus is probably an *Exosporium*. Other differences were noted. The conidia and conidiophores of the juniper fungus are decidedly shorter than those of the arborvitae fungus. The juniper fungus was obtained in culture by isolating single conidia and compared with cultures of the arborvitae fungus. Cultures of the two fungi on the same medium were distinctly different.

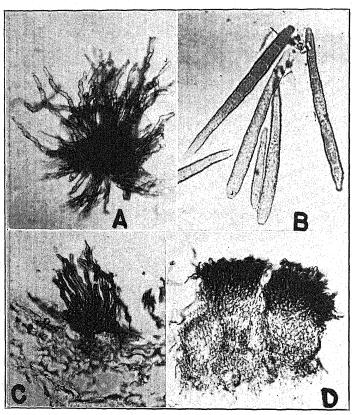


Fig. 3. A. Cercospora thujina. Sporophore fascicle pressed between glass cover slips. ×180. B. Cercospora thujina conidia greatly enlarged to show the echinulation. ×780. C. Section through fructification of C. thujina showing that the conidiophores arise from a thin stromatic base (compare with D). ×180. D. Section through fructification of Cercospora Sequeiae var. Juniperi showing the thick, sporodochium-like sclerotial stroma from which arise short conidiophores. ×180.

The arborvitae fungus agrees somewhat with the descriptions of *Cercospora Sequoiae* in respect to the size, shape, and color of its conidiophores and conidia. However, the following statement in Ellis and Everhart's description of *C. Sequoiae* suggests that the fruiting body of this fungus, notwithstanding its longer conidiophores, is probably similar to that of *C. Sequoiae* var. *Juniperi*: "Forming large, compact, olivaceous tufts which, under lens,

resemble perithecia of *Sphaerella*." The fruiting bodies of the arborvitae fungus do not resemble perithecia when viewed under lens. They consist of fascicles of spreading conidiophores originating from a narrow base.

Georgescu and Badea (4) described Cercospora juniperina on Juniperus communis in Roumania, although in an earlier publication (3), these authors had named their fungus Camarosporium juniperinum. They described the conidia as echinulate. Later, Sandu-Ville (7) collected and examined specimens of the fungus described by Georgescu and Badea and reported that the fungus was neither a Camarosporium nor a Cercospora but an Exosporium, probably E. deflectens Karst.

Dr. Charles Chupp, who kindly examined specimens of the arborvitae fungus submitted to him, found the conidia echinulate like those of C. Sequoiae and C. Sequoiae var. Juniperi which he had examined earlier. At that time, Dr. Chupp was of the opinion that these three fungi were either identical, or very closely related, and, in a letter, expressed the opinion that they did not fit in the genus Cercospora, first because of the echinulation of their conidia, and secondly because of the promiscuity in their host relationship.

The arborvitae fungus is apparently distinct from those on Sequoia and Juniperus. Furthermore, it is doubtful that strict host specificity in the case of Cercospora has been proven unquestionably. The objection regarding the echinulations of the conidia is more valid because no provision is made in the description of the genus Cercospora for inclusion of forms with echinulate spores. In the case of the arborvitae fungus (also of those on Sequoia and Juniperus) the echinulations are so faint and indistinct that fairly high magnification is necessary (Fig. 3, B) to see them. In fact, Ellis and Everhart apparently failed to note the echinulation, for they made no mention of it in their description of C. Sequoiae and C. Sequoiae var. Juniperi. In all other characters, the arborvitae fungus fits well in the genus Cercospora, and it is therefore described as a new species under the name of C. thujina, n. sp.

Description

The fungus fructifies fairly abundantly on recently killed leaves and branchlets, producing grayish tufts of conidiophores which, when examined with a dissecting microscope, have the outline of shrubs with relatively narrow bases and spreading branches. The conidiophores (Fig. 3, A) which are very numerous in each fascicle (as many as 50 have been counted) arise from a relatively thin, more or less disc-like stromatic base. They are long, flexuous, sparingly septate, rarely branched, somewhat irregular in diameter, yellowish-brown, with several spore scars. The conidia (Fig. 3, B) are oblong, tapering, with obconic-truncate bases, of approximately the same color as the conidiophores, septate, faintly echinulate.

The fungus grows easily but slowly in culture. The colonies vary from dark greenish gray on some media (bean-pod agar for example) to light

gray on others (potato-dextrose, corn-meal and oatmeal agars). Patches (not sectors) of whitish aerial growth often develop on the colonies. Spores were never observed in culture, although many attempts were made to induce sporulation by growing the fungus under different environmental conditions on a variety of media, such as bean-pod, oatmeal, corn-meal, potato-dextrose, and Czapek agars, and on autoclaved bean pods, potato (white and sweet) plugs, and arborvitae twigs either alone or with the addition of soil to the flasks.

Cercospora thujina, n. sp.

Conidiophores in dense fascicles arising from a relatively thin, disc-like, stromatic base, long, flexuous, sparingly septate, irregular in diameter, yellowish brown, with several spore scars, $26.0-105\times3.3-5.0\,\mu$ (average $55.8\times4.3\,\mu$); conidia oblong, tapering, straight or slightly curved, with obconic-truncate bases, yellowish brown, 1–7 septate (average number of septa 4.2), faintly echinulate, 19.0–56.0 \times 4.9–6.6 μ (average 42.2 \times 5.5 μ). Parasitic on *Thuja orientalis* L. causing necrosis of leaves and branchlets and

Type locality: Baton Rouge, Louisiana, U. S. A.

Type specimens deposited in the herbarium of the Department of Botany, Louisiana State University, under accession No. 4677, and also in the Mycological Collections, Bureau of Plant Industry, Washington, D. C., and in the Farlow Herbarium, Harvard University.

Maculis indeterminatis, brunneis; conidiophoris in fasciculis densis e basi stromatica, tenui planaque oriundis, longis, flexuosis, parce septatis, crassitudine inaequalibus, fulvis, cicatrices aliquot conidiis factas habentibus, 26.0-105 x 3.3-5.0 µ; conidiis oblongis, attenuatis, rectis vel subcurvatis, bases obconico-truncatas habentibus, fulvis, 1-7 septatis, minute echinulatis, 19.0–56.0 $\times 4.9$ –6.6 μ

Hab. Ad Thuja orientalem L., ut folia ramulique moriantur efficiens. Baton Rouge,

Louisiana, Amer. bor.

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CONTROL

In the summer of 1943, a planting of arborvitae containing 107 diseased plants (59 Baker and 48 Berckman's Golden) in a nursery in Baton Rouge was divided into 4 sections and treated as follows:

Section I. 26 plants. Check, not treated.

Section II. 28 plants. Sprayed with 4-4-50 Bordeaux.

Section III. 27 plants. Sprayed with Cuprocide (89 per cent Cu) at the rate of 3 lb. in 100 gallons of water.

Section IV. 26 plants. Sprayed with Tennessee Copper Co. "Tribasic" copper sulphate (53 per cent Cu) at the rate of 6 lb. in 100 gallons of water.

Four spray applications were made, on June 15, July 19, August 4, and September 3.

Since the severity of the disease varied, each plant was examined carefully at the start of the experiment, and marked light, medium, or severe. This was a rough classification based on gross observation, but proved helpful in evaluating the results of the experiment.

All 3 sprays gave practically complete control. The progress of the disease on the sprayed plants was completely checked, and the new growth developing after spraying remained healthy. There was no shedding of branchlets. In the nonsprayed check plants, on the other hand, the disease continued to spread. The little new growth that developed became diseased. By the end of October, the ground under the nonsprayed plants was littered with fallen diseased branchlets. Two plants (both Berckman's Golden) were completely killed. Many of the plants whose disease condition was marked light at the start of the experiment were in the medium or severe classes by October.

The contrast between the sprayed and nonsprayed plants was most striking in a section of 21 plants of the Baker variety. At the start of the test, all were classed as light in respect to disease. Eleven were sprayed with 4-4-50 Bordeaux and the other 10 were left as checks. By the end of October, the check plants were classed as severe; there was considerable spread of the blight upward and much shedding of necrotic branchlets. The sprayed plants, on the other hand, were practically normal; the progress of the disease was completely stopped, and the new growth remained healthy.

Although more work must be done to determine the optimum time to spray, the minimum number of applications for effective control, etc., the results of this one experiment were clear-cut enough to show that probably the disease can be controlled by spraying.

The Bordeaux spray left a rather heavy deposit on the plants which might be considered objectionable until toned down by weathering, although from a distance the Bordeaux-sprayed plants appeared deeper green and healthier. The Cuprocide imparted a light green, slightly rusty hue to the plants, but this was not prominent enough to be objectionable. The Tribasic spray residue, being greenish-blue, was hardly visible even at close inspection.

After this paper was prepared for publication, J. A. Pinckard brought to the writer's attention a publication (5) in which it is reported that, although the cause of the disease was not known, effective control was obtained in Mississippi by three separate agencies working independently. Copper sprays were used by all three. T. G. Owen and Son, nurserymen, Columbus, Mississippi, were the first to control the disease by spraying with Cuprocide. Paul V. Siggers, Division of Forest Pathology, U. S. Department of Agriculture, obtained control by using 4–4–50 Bordeaux at approximately monthly intervals. M. L. Grimes and J. A. Pinckard used 3 different copper sprays, 4–4–50 Bordeaux, Cuprocide, and basic copper sulphate, the last two at 3 lb. per 100 gallons of water. All 3 sprays gave satisfactory control.

SUMMARY

A destructive disease of oriental arborvitae, known locally as "blight" or "fire," is characterized by necrosis and shedding of foliage and branchlets, and by the formation of bark cankers on small twigs.

The cause of the disease, demonstrated by pure culture inoculations, is a *Cercospora*, apparently an undescribed species. This is described under the name of **C.** thujina, n. sp.

Perithecia of *Mycosphaerella sp.* often were found on diseased arborvitae leaves and branchlets, either alone or in association with the *Cercospora*, and it was thought at first that this might be the ascigerous stage of the latter.

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Repeated inoculations with several ascospore isolates of the Mycosphaerella, however, failed to cause infection, and it must be assumed, therefore, that this fungus bears no relation either to the Cercospora or to the disease.

A limited spraying test using 4-4-50 Bordeaux, cuprous oxide (Cuprocide, 89 per cent Cu) at the rate of 3 lb. in 100 gallons of water, and basic copper sulphate (Tennessee Copper Co. "Tri-basic," 53 per cent Cu) at the rate of 6 lb. in 100 gallons of water, checked completely the progress of the disease on diseased plants. These results indicate that the disease is amenable to control by copper.

DEPARTMENT OF PLANT PATHOLOGY, LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE, LOUISIANA.

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EFFECT OF SEED TREATMENT ON PROTECTION, RATE OF EMERGENCE, AND GROWTH OF GARDEN PEAS

L. D. LEACH AND PAUL G. SMITH 2

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The beneficial effects of certain mercury and copper compounds in reducing seed and seedling rots of peas have been known and used commercially for a number of years. Several authors have contributed information on the factors that influence pea seed decay and on the relative effectiveness of different fungicides. This information was summarized by Walker *et al.* (23) in 1940.

Recently several nonmetallic fungicides have been introduced and found to be highly beneficial as pea seed protectants. Additional information on the evaluation of both metallic and nonmetallic fungicides has been published by Sharvelle and Shema (19), McNew (12, 15, 16), Felix (6), Sharvelle et al. (20), and by Bayliss et al. (4). The cooperative tests conducted in 1940, 1941, 1942, and 1943, by the Committee for Coordination in Cereal and Vegetable Seed Treatment Research of the American Phytopathological Society have added valuable information on the use of seed treatments on several varieties of peas and in different parts of North America.

The soil organisms responsible for seed decay and seedling rot have been studied by several investigators (3, 7, 9, 10, 17, 18). Most authors concluded that species of *Pythium* were the principal causes of the disease. Some found that species of *Rhizoctonia*, *Phytophthora*, *Fusarium*, and *Botrytis* caused high mortality of pea seeds and seedlings. Most tests have been in naturally infested soils and information on the relative protective value of fungicides on pea seed against specific organisms is limited. In one of the few trials with specific organisms, McNew (13) tested red and yellow cuprous oxide, Semesan, and Spergon on peas in soil infested with *Pythium ultimum* and found them equally effective as protectants.

Injurious effects upon peas have been reported from seed treatment with copper, mercury, and zinc compounds. Horsfall et al. (8) reported that red copper oxide occasionally caused slight stunting, especially in soil deficient in organic matter, while Kadow and Anderson (12) observed some injury on Perfection and Wisconsin Early Sweet varieties. Cook (5) found that zinc oxide tended to stunt the pea plants. From observations and yield data on field plantings McNew (12) concluded that cuprous oxide may cause slight injury to peas under certain conditions. In the same trials he found that New Improved Ceresan injured Surprise, Green Admiral, and Wisconsin Early Sweet varieties and concluded that this fungicide was too injurious for use on peas.

Experiment Station, University of California.

² Instructor in Truck Crops and Junior Olericulturist in the Experiment Station, University of California.

¹ Associate Professor of Plant Pathology and Associate Plant Pathologist in the Experiment Station, University of California.

Increased yields from treated pea seeds have led to discussions as to whether the increases could be attributed entirely to disease control or whether they were produced in part by chemical stimulation. Horsfall (7) found that red copper oxide accelerated emergence of pea seeds from cold wet soil infested with Pythium ultimum. He also observed other evidences of stimulation on vegetable crops but concluded that some of the differences might be due to density of stand or freedom from disease, although the possibility of response to copper as a nutritional element was not excluded. Concerning this point Kadow and Anderson (10) stated that "the benefit from treated pea seed is not limited to damping off control but extends to the general welfare of the entire plant." The same authors report that treatment of pea seed may materially reduce stem and root rots, and also refer (1) to the importance to seedling vigor of protecting cotyledons against decay. It appears, therefore, that the additional benefits from seed treatment were due, chiefly, to disease control rather than chemical stimulation. After testing Spergon on canning peas in Minnesota, Sharvelle and Shema (19) reported that in addition to its protective value it appeared to stimulate growth, and in a later paper Sharvelle et al. (20) claimed that treating seed with Spergon increased the rate of emergence and produced greater vine length and more vigorous root development. It is significant that their comparisons were between Spergon-treated and nontreated seeds in field plots and, except in a few trials, not between different seed treatments.

The most direct evidence of chemical stimulation by Spergon was supplied by McNew (13, 15), who found that disease-free pea seed planted in steamed soil produced 5 to 20 per cent more dry matter when treated with Spergon than when nontreated or treated with other fungicides, and in another report (14) stated that Spergon "as a growth stimulant . . . should pay dividends in practically every field, irrespective of growth conditions." In a repetition of his earlier trials, however, McNew secured no significant differences in the seedling weights from various seed treatments in steamed soil.

The present investigations were to determine (1) the relative protective value of several metallic and nonmetallic compounds under controlled conditions with known organisms and under field conditions, and to determine (2) whether under local conditions any of these materials produced injurious or beneficial growth effects aside from protection from decay organisms.

MATERIALS AND METHODS

The experiments were in three series: (1) greenhouse trials with sterilized soil inoculated with cultures of *Pythium ultimum* or *Rhizoctonia solani*, (2) field plantings at various seasons of the year, and (3) greenhouse trials in sterilized soil.

For treatment, the required amount of seed was weighed out and placed in 1-quart fruit jars provided with a wooden baffle to insure adequate mixing ³ Studies on Vegetable Seed Treatments in 1943. Plant Disease Reporter, Supple-

ment 145: 11. 1944.

of the seed. After adding the desired quantity of chemical, these jars were rotated on a motor-driven device until the seeds were thoroughly coated with dust. Since these jars had been used previously for the same seed treatment, they were thoroughly coated on the inside with the particular chemical used and very little loss or addition of dust was to be expected.

The soil used in the tests with specific organisms was first pasteurized by passing it through the electrically heated device described by Tavernetti (21). In repeated trials this apparatus has eliminated the common damping-off fungi from soils when proper temperature and soil moisture are maintained. After pasteurization the soils were infested with either Pythium or Rhizoctonia by adding agar cultures of the fungus. A Waring Blendor was used to suspend the agar cultures in sterile water, as suggested by Andrus (2). The fungus suspension was mixed with the pasteurized soil and incubated for 5 days at about 20° C. before the seeds were planted.

PROTECTION AGAINST SPECIFIC ORGANISMS

In previously sterilized soil thoroughly infested with Pythium ultimum, greenhouse trials were run in the winter of 1942 and 1943. The experiments consisted respectively of 5 and 6 randomized replicates with 15 seeds for each plot in each replicate. The soil was well moistened before planting, and prevented from drying by a light watering as needed. Daily counts were made during the period of emergence. About 4 weeks after planting, the plants were dug, washed, and inspected. In order to express the severity of infection by a single figure, the following system of rating was used: no infection, 0; one cotyledon decayed, or slight damage, 25; both cotyledons decayed or moderate damage, 50; post-emergence damping-off, 75; and preemergence damping-off, 100. The disease index for each plot was obtained by multiplying each class value by the number of plants in that class, adding the products, and dividing the total by the number of seeds planted in each plot. A slight error is introduced by non-viable seeds being classed as preemergence damping-off but with high germinating seed this error would be small and about equal in all treatments.

In 1942 all five treatments gave a highly significant increase in emergence and freedom from infection as compared with the nontreated seed (Table 1). Semesan and Yellow Cuprocide provided the best protection with Spergon, New Improved Ceresan, and Arasan apparently somewhat less effective. The few nontreated seedlings that emerged had cotyledon rot or epicotyl infection, and small stunted plants resulted. The average weight of green seedlings (Table 1) has an inverse relation to the disease index. When the cotyledons were removed, seedling weights were more nearly equal for the various treatments.

In 1943 all treatments were again much better than the control but between treatments most of the differences in emergence were nonsignificant and with respect to the disease index they are of doubtful significance. As in 1942 seedling weight was highest after treatment with Semesan and Yellow Cuprocide but this advantage was due largely to the weight of cotyledons (Table 1). This is an indication of the high protective value of these two chemicals against Pythium infection but trials in sterile soil, reported later in this paper, show, in addition, evidence of direct chemical effects upon the cotyledons.

The two years' experiments in soil infested with *Pythium ultimum* indicate that against this particular organism, Yellow Cuprocide and Semesan

TABLE 1.—Relation of pea seed treatments, on the variety Laxton's Progress, to protection and growth in soil infested with Pythium ultimum

Planted in greenhouse Dec. 16, 1942

		Averag	e weight per	t per seedling		
	Danama	Emer-	Disease	G	Drya	
Treatment	Treatment Dosage gen		index	With coty-ledons	Coty- ledons removed	Coty- ledons removed
	Per cent	Per cent		grams	grams	grams
NontreatedSpergonNew Improved	0.187	$\begin{array}{c} 5.6 \\ 72.2 \end{array}$	97.8 36.6	0.68 ^b 1.58	0.50b 1.10	0.035b 0.117
Ceresan Yellow Cuprocide Semesan Arasan	0.094 0.187 0.312 0.187	78.9 92.2 92.2 51.1	$38.9 \\ 12.0 \\ 11.4 \\ 63.6$	1.54 1.73 1.76 1.35	1.10 1.18 1.28 1.01	0.118 0.118 0.124 0.115
	Odds 19:1 Odds 99:1	$\begin{array}{c} 12.1 \\ 16.3 \end{array}$	11.8 15.9	$0.18 \\ 0.24$	0.17 n.s.c	n.s. n.s.
Planted in greenhouse	Nov. 26, 194	3				
Nontreated Spergon New Improved	0.187	17.3 66.7	88.0 44.7	0.84 ^b 1.83	0.60b 1.64	$0.08^{\rm b} \ 0.162$
Ceresan Yellow Cuprocide Semesan Arasan	$0.094 \\ 0.156 \\ 0.25 \\ 0.187$	73.4 78.7 70.7 74.7	39.3 28.3 35.0 39.7	1.95 2.16 2.14 1.56	1.69 1.74 1.77 1.36	0.166 0.157 0.169 0.138
	Odds 19:1 Odds 99:1	13.7 18.7	13.6 18.5	$\begin{array}{c} 0.26 \\ 0.36 \end{array}$	0.20 0.30	$0.018 \\ 0.024$

a Dried to constant weight at 70° C.

c Differences not significant.

gave the most effective protection. Spergon, New Improved Ceresan, and Arasan were only a little less effective.

Recently ter Horst and Felix (22) reported the high fungicidal potency of the compound "604" (2,3,dichloro-1,4-naphthoquinone) as a protectant for peas. During the present investigations, in a single greenhouse test in soil naturally infested with *Pythium ultimum*, the percentages of emergence were as follows: Nontreated, 11; "604" at a dosage of 0.2 per cent, 79; "604" at 0.1 per cent, 67; "604" at 0.05 per cent, 54; Spergon at 0.2 per cent, 47; and Semesan at 0.25 per cent, 82.

b Not included in statistical analysis because of small number of surviving plants.

These results indicate that the new compound "604" is highly effective as a protectant for peas in soil infested with *Pythium*.

Similar trials were made with soil infested with an isolate of *Rhizoctonia* solani pathogenic on bean and beet seedlings but not tested on peas. In two experiments there was little pre-emergence damping-off, perhaps because conditions were unfavorable for infection. As a result there were no significant differences in emergence between nontreated and treated lots. Post-

TABLE 2.—Relative protection, rate of emergence, and dry seed yield resulting from seed treatments in field plantings
Variety Laxton's Progress. Planted Jan. 14, 1943

Per cent Per cent Days Grams Grams Spergon 0.187 85.6 25.4 651.0 7.58	Treatment	Dosage	Emer-	Mean emer-		Average yield of dry seed	
Nontreated			genee	0	Per plot	Per plant	
Spergon		Per cent	Per cent	Days	Grams	Grams	
New Improved Ceresan 0.094 82.8 25.9 593.0 7.18 Yellow Cuprocide 0.187 83.0 32.0 565.5 6.82 Semesan 0.312 88.3 26.4 634.5 7.18 Significant difference Odds 19: 1 5.6 2.4 48.4 0.75 Odds 99: 1 7.6 3.3 66.0 1.02 Variety Wisconsin Early Sweet. Planted Jan. 14, 1943 Nontreated 18.5 24.8 185.2 10.01 Spergon 0.187 55.1 28.0 451.2 8.14 New Improved Ceresan 0.094 41.8 30.4 339.3 8.19 Yellow Cuprocide 0.187 43.0 30.8 274.5 6.37 Significant difference Odds 19: 1 5.0 1.4 61.2 1.31 Odds 99: 1 6.7 1.9 83.4 1.79 Variety Laxton's Progress. Planted Aug. 16, 1943 Nontreated 29.3 8.95	Nontreated	***************************************	58.3	26.6	532.7	9.14	
Yellow Čuprocide 0.187 83.0 32.0 565.5 6.82 Semesan 0.312 88.3 26.4 634.5 7.18 Significant difference Odds 19: 1 5.6 2.4 48.4 0.75 Odds 99: 1 7.6 3.3 66.0 1.02 Variety Wisconsin Early Sweet. Planted Jan. 14, 1943 Nontreated 18.5 24.8 185.2 10.01 Spergon 0.187 55.1 28.0 451.2 8.14 New Improved Ceresan 0.094 41.8 30.4 339.3 8.19 Yellow Čuprocide 0.187 43.0 30.8 274.5 6.37 Semesan 0.312 58.0 28.4 441.7 7.62 Significant difference Odds 19: 1 5.0 1.4 61.2 1.31 Odds 99: 1 6.7 1.9 83.4 1.79 Variety Laxton's Progress. Planted Aug. 16, 1943 Nontreated 29.3 8.95 Spergon 0.187 64.8 9.48 New Improved Ceresan 0.094	Spergon	0.187	85.6	25.4	651.0	7.58	
Semesan	New Improved Ceresan	0.094	82.8	25.9	593.0	7.18	
Significant difference Odds 19: 1	Yellow Cuprocide			32.0	565.5	6.82	
Variety Wisconsin Early Sweet. Planted Jan. 14, 1943	Semesan	0.312	88.3	26.4	634.5	7.18	
Variety Wisconsin Early Sweet. Planted Jan. 14, 1943 Nontreated 18.5 24.8 185.2 10.01 Spergon 0.187 55.1 28.0 451.2 8.14 New Improved Ceresan 0.094 41.8 30.4 339.3 8.19 Yellow Cuprocide 0.187 43.0 30.8 274.5 6.37 Semesan 0.312 58.0 28.4 441.7 7.62 Significant difference Odds 19: 1 5.0 1.4 61.2 1.31 Odds 99: 1 6.7 1.9 83.4 1.79 Variety Laxton's Progress. Planted Aug. 16, 1943 Nontreated 29.3 8.95 Spergon 0.187 64.8 9.48 New Improved Ceresan 0.094 56.5 8.88 Yellow Cuprocide 0.187 70.7 9.12 Semesan 0.312 81.0 9.50 Significant difference Odds 19: 1 7.9 n.s.a	G: : C	Odds 19:1	5.6	2.4	48.4	0.75	
Nontreated	Signineant dinerence	Odds 99:1	7.6	3.3	66.0	1.02	
Spergon 0.187 55.1 28.0 451.2 8.14 New Improved Ceresan 0.094 41.8 30.4 339.3 8.19 Yellow Cuprocide 0.187 43.0 30.8 274.5 6.37 Semesan 0.312 58.0 28.4 441.7 7.62 Significant difference Odds 19: 1 5.0 1.4 61.2 1.31 Odds 99: 1 6.7 1.9 83.4 1.79 Variety Laxton's Progress. Planted Aug. 16, 1943 Nontreated 29.3 8.95 Spergon 0.187 64.8 9.48 New Improved Ceresan 0.094 56.5 8.88 Yellow Cuprocide 0.187 70.7 9.12 Semesan 0.312 81.0 9.50 Significant difference Odds 19: 1 7.9 n.s.²	Variety Wisconsin Early	Sweet. Plant	ed Jan. 14,	1943			
New Improved Ceresan 0.094 41.8 30.4 339.3 8.19 Yellow Cuprocide 0.187 43.0 30.8 274.5 6.37 Semesan 0.312 58.0 28.4 441.7 7.62 Significant difference Odds 19: 1 5.0 1.4 61.2 1.31 Odds 99: 1 6.7 1.9 83.4 1.79 Variety Laxton's Progress. Planted Aug. 16, 1943 1.79 1.8 Nontreated 29.3 8.95 8.8 8.8 New Improved Ceresan 0.094 56.5 8.88 8.8 Yellow Cuprocide 0.187 70.7 9.12 9.12 Semesan 0.312 81.0 9.50 8.8 Significant difference Odds 19: 1 7.9 n.s.a 7.9	Nontreated		18.5	24.8	185.2	10.01	
Yellow Cuprocide 0.187 43.0 30.8 274.5 6.37 Semesan 0.312 58.0 28.4 441.7 7.62 Significant difference Odds 19: 1 5.0 1.4 61.2 1.31 Significant difference Odds 99: 1 6.7 1.9 83.4 1.79 Variety Laxton's Progress. Planted Aug. 16, 1943 Nontreated 29.3 8.95 8.95 Spergon 0.187 64.8 9.48 8.88 Yellow Cuprocide 0.187 70.7 9.12 9.12 9.50 Semesan 0.312 81.0 9.50 9.50 8.88 Significant difference Odds 19: 1 7.9 n.s.a 9.50 9.50	Spergon	0.187	55.1	28.0	451.2		
Semesan 0.312 58.0 28.4 441.7 7.62 Significant difference Odds 19: 1 5.0 1.4 61.2 1.31 Odds 99: 1 6.7 1.9 83.4 1.79 Variety Laxton's Progress. Planted Aug. 16, 1943 Nontreated 29.3 8.95 Spergon 0.187 64.8 9.48 New Improved Ceresan 0.094 56.5 8.88 Yellow Cuprocide 0.187 70.7 9.12 Semesan 0.312 81.0 9.50 Significant difference Odds 19: 1 7.9 n.s.2	New Improved Ceresan						
Significant difference Odds 19: 1	Yellow Cuprocide						
Significant difference Odds 99: 1 6.7 1.9 83.4 1.79 Variety Laxton's Progress. Planted Aug. 16, 1943 Nontreated 29.3 8.95 Spergon 0.187 64.8 9.48 New Improved Ceresan 0.094 56.5 8.88 Yellow Cuprocide 0.187 70.7 9.12 Semesan 0.312 81.0 9.50 Significant difference Odds 19: 1 7.9 n.s.a	Semesan	0.312	58.0	28.4	441.7	7.62	
Variety Laxton's Progress. Planted Aug. 16, 1943 Nontreated 29.3 8.95 Spergon	C:	Odds 19:1	5.0	1.4	61.2	1.31	
Nontreated 29.3 8.95 Spergon 0.187 64.8 9.48 New Improved Ceresan 0.094 56.5 8.88 Yellow Cuprocide 0.187 70.7 9.12 Semesan 0.312 81.0 9.50 Significant difference Odds 19: 1 7.9 n.s.2	Signincant difference	Odds 99:1	6.7	1.9	83.4	1.79	
Spergon 0.187 64.8 9.48 New Improved Ceresan 0.094 56.5 8.88 Yellow Cuprocide 0.187 70.7 9.12 Semesan 0.312 81.0 9.50 Significant difference Odds 19: 1 7.9 n.s.a	Variety Laxton's Progre	ss. Planted A	ug. 16, 194	3			
Spergon 0.187 64.8 9.48 New Improved Ceresan 0.094 56.5 8.88 Yellow Cuprocide 0.187 70.7 9.12 Semesan 0.312 81.0 9.50 Significant difference Odds 19: 1 7.9 n.s.a	Nontreated		29.3	8.95			
New Improved Ceresan 0.094 56.5 8.88 Yellow Cuprocide 0.187 70.7 9.12 Semesan 0.312 81.0 9.50 Significant difference Odds 19: 1 7.9 n.s.a	Spergon	0.187	64.8	9.48			
Semesan 0.312 81.0 9.50 Significant difference Odds 19:1 7.9 n.s.a	New Improved Ceresan	0.094	56.5	8.88			
Significant difference Odds 19:1 7.9 n.s.2	Yellow Cuprocide	0.187	70.7		***********		
Significant difference	Semesan	0.312	81.0	9.50			
	C: 10 / 7100	Odds 19:1	7.9	n.s.a	,,,,,,,,,,		
	Significant difference		10.8	n.s.	**********		

a Difference not significant.

emergence damping-off, however, was severe in all cases. Inspection of the seedlings showed a high degree of cotyledon rot and stem rot, regardless of the treatment. In these experiments, none of the materials was effective in controlling cotyledon rot and post-emergence rot caused by *Rhizoctonia*. Additional trials would be necessary to determine to what extent these results were influenced by the strain of the fungus or by environmental conditions

FIELD EXPERIMENTS

The field experiments involved four different planting dates and five varieties of peas. The January and August plantings (Table 2) represent

the approximate weather conditions encountered during the two commercial pea planting seasons in the interior valleys of California. The February and March plantings (Table 3), conducted as part of the cooperative tests arranged by the Committee on Coordination of Cereal and Vegetable Seed Treatment Research of the American Phytopathological Society, represent periods too late for the spring pea crop.

In addition to the usual data on emergence and yield the writers have found the rate of emergence to be an excellent indicator of the effect of seed treatment. The most satisfactory measure of the emergence rate is the coefficient of velocity of emergence proposed by Kotowski (11). The first requisite is that the emergence stand be counted frequently during the germination period, preferably at daily intervals. Each daily increase in emergence is multiplied by the number of days since planting; the sum of these products is divided into the total emergence at the end of the trial and the quotient is then multiplied by 100 to give the coefficient of velocity of emergence.

The rate of emergence can be measured also by the mean emergence period, calculated in the same way as the coefficient of velocity except that the sum of the products is divided by the total emergence at the end of the trial. Also, the mean emergence period can be determined by dividing the coefficient of velocity into 100 since the former term is the reciprocal of the latter times 100.

The mean emergence period represents the weighted mean time required for the emergence of all seedlings for the treatment. Such a figure is obviously influenced by the seed, the depth of planting, moisture, temperature, and seed treatment. Within any one experiment all but the last factor can be assumed to be reasonably uniform. The writers have found the mean emergence period to be a more useful unit than the coefficient of velocity of emergence because differences between treatments in the same experiment represent the actual number of days by which the mean germination of one lot was delayed as compared to another lot.

The January planting in 1943 consisted of two varieties: Laxton's Progress and Wisconsin Early Sweet each tested with nontreated seed and four seed treatments. Each treatment was replicated six times and each plot consisted of a single row planted with 100 seeds. Between planting and emergence 7.8 inches of rain fell. The air temperature varied from a minimum of 21° F. to a maximum of 62° F. with a mean of 47° F., while the soil temperature at the depth of planting varied from 32° F. to 57° F. with a mean of 45.5° F. The first seedlings emerged 21 days after planting. In emergence of Laxton's Progress, there was no significant difference between the treatments (Table 2) but all were very much better than the nontreated control. Isolations from nontreated pea seeds removed from the soil before emergence indicate that *Pythium ultimum* was the pathogen chiefly responsible for pre-emergence damping-off in the early spring plantings. The data on mean emergence period (Table 2, column 4) show clearly that Yellow

Cuprocide delayed emergence by at least five days as compared with non-treated seed or with other treatments. The yields suggest that Spergon and Semesan combined excellent protection with freedom from injurious effects while the high yield per plant and low yield per plot in the control is undoubtedly due to the low density of stand. Yellow Cuprocide, in addition to delaying emergence, reduced the yield per plant and yield per plot.

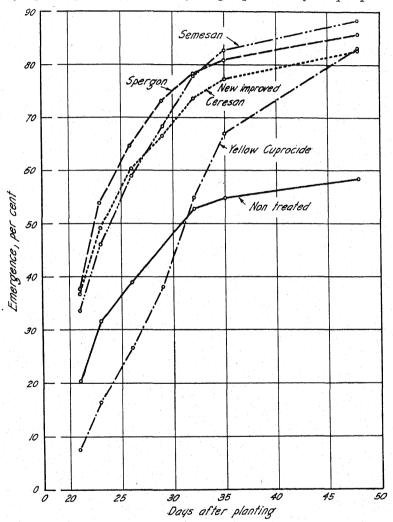


Fig. 1. Emergence curves for Laxton's Progress peas treated with seed protectants and planted at a low temperature. All treatments gave adequate protection but Yellow Cuprocide delayed emergence.

With Wisconsin Early Sweet the highest emergence was produced by Spergon and Semesan although all treatments produced much better stands than the control. Both Yellow Cuprocide and New Improved Ceresan appeared to delay emergence as compared with the other treatment and the

lower emergence from these treatments may therefore be due either to lower protective value or to injurious effects.

The rapid emergence of nontreated seed suggests that all treatments delayed germination but reference to the emergence curves (Figures 2 and 3) suggests another explanation. Apparently pre-emergence damping-off

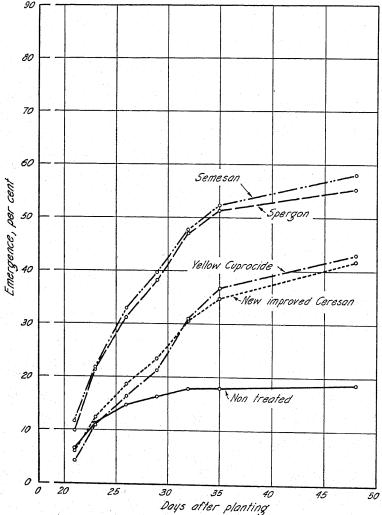


Fig. 2. Emergence curves for Wisconsin Early Sweet garden peas treated with seed protectants and planted at low temperature. The emergence rates were influenced by both protective effects and chemical injury.

was so severe that only the early germinating plants emerged, while most of the late germinating seed rotted below the surface of the soil. By contrast treatment of the seed provided protection for the late germinating seed. It is obvious, therefore, that comparisons between rates of emergence with different treatments should be limited to plantings in noninfested soil or to comparisons of treatments that provide nearly equal protection.

Because of low density of stand the nontreated control again had the highest yield per plant but the lowest yield per plot. The low per plant yield from seed treated with Yellow Cuprocide again suggests chemical injury while the yields from the other three treatments were in proportion to the density of stand.

The August planting of Laxton's Progress was made in the period during which the seed for the fall crop of peas is planted in Central California. In contrast to the low temperature that prevails during the spring plantings,

TABLE 3.—Effect of seed treatment on stand and yield of garden peas. These data were obtained as part of the cooperative trials for the Committee for Coordination in Cereal and Vegetable Seed Treatment Research

Varieties Surprise and Alderman. Planted Mar. 18, 1942

		Surprise		Alderman		
Treatment	Dosage			Green weighte		
		Emergence	Emergence	Vines and pods	$_{ m Pods}$	
	Per cent	Per cent	Per cent	Grams	Grams	
Nontreated	0.279 0.168 0.279 0.223 Odds 19: 1 Odds 99: 1	42.2 74.8 79.4 84.2 83.0 7.8 10.8	34.8 83.0 85.4 87.4 83.4 7.8 10.8	672 1528 1628 1614 1554 310 427	222 534 554 496 531 93 128	
Nontreated Arasan Fermate Spergon	a a a		76.2 85.1 83.3 86.6	1930 2400 2347 2400	1120 1374 1344 1355	
Significant difference	Odds 19: 1 Odds 99: 1		5.26b 7.06	310 ^b 420	n.s.	

^a Emergence and yields based on averages for 3 dosages. Arasan, Fermate, and Spergon were each used at the following dosages: 0.084 per cent, 0.168 per cent, and 0.335 per cent. Differences between dosages and interaction of dosage and treatment were non-significant.

soil temperatures are high and high soil moisture is provided by irrigation either before or after planting. It is also very probable that different seed decay and damping-off organisms are active at this time of the year. In this trial the soil temperature at the depth of planting varied from a minimum of 57° F. to a maximum of 94° F. with a mean of 73.4° F. The first seedlings emerged on the sixth day. Only stand and rate of emergence records were taken. The best stand was secured from Semesan-treated seed followed by Yellow Cuprocide, Spergon, and New Improved Ceresan in that order. Under the high temperature prevailing, the germination of all lots was comparatively rapid and none of the treatments appeared to depress the rate of emergence.

b Differences required for significance between treatment means and nontreated; differences between treatment means nonsignificant.

c Yields for this experiment are very low because of the late planting date.

Seed treatments on three additional varieties are compared in table 3. On all three varieties, Surprise, Alderman, and Thomas Laxton, each seed treatment produced a significant increase in stand over the control but there were no significant differences in stand between treatments except that Red Cuprocide appeared to be less effective on Surprise than Semesan or Spergon. On both Alderman and Thomas Laxton all treatments increased the yield as compared with nontreated seed but there were no significant differences between treatments.

The fact that the three different chemicals used in the 1943 planting produced nearly equal increases in yield indicates that the result was due to protective values and not to the specific stimulating effects of any of the chemicals.

TRIALS IN PASTEURIZED SOIL IN THE ABSENCE OF DISEASE

To measure the effect of seed treatments on the rate of emergence and growth of pea seedlings in the absence of disease two greenhouse plantings were made in soil that had been pasteurized to eliminate pathogenic fungi.

In the first trial, seed treated with Spergon and New Improved Ceresan

TABLE 4.—Rate of emergence and plant growth in sterile soil of treated pea seed, Variety Laxton's Progress, grown in flats in the greenhouse Planted Jan. 15, 1943

				Average weight per seedling				
Treatment Dos.	Dosage	Emer-	Mean emer-	Green plants			Dried plants ^a	
	gence gence	gence period	With coty- ledons	Coty- ledons removed	Coty- ledons only	Coty- ledons removed		
	$Per \ cent$	Per cent	Days	Grams	Grams	Grams	Grams	
Nontreated Spergon New Improved	0.187	97.4 95.4	$\frac{6.90}{7.00}$	$2.49 \\ 2.54$	2.04 2.04	$\begin{array}{c} 0.45 \\ 0.50 \end{array}$	$0.156 \\ 0.162$	
Ceresan	0.094	97.4	7.12	2.52	2.05	0.47	0.159	
Significant difference	Odds 19:1 Odds 99:1	n.s.b n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Planted Dec. 3, 19)43							
Nontreated Spergon New Improved	0.187	93.4 90.0	8.71 8.78	2.33 2.44	2.11 2.16	0.227 0.276	0.159 0.158	
Ceresan Yellow	0.094	86.7	8.56	2.36	2.12	0.246	0.158	
Cuprocide Semesan	$0.156 \\ 0.25 \\ 0.187$	90.7 97.4 92.7	9.39 8.85 8.59	2.17 2.36 2.35	1.85 2.04 2.11	$0.328 \\ 0.320 \\ 0.235$	$0.143 \\ 0.145 \\ 0.159$	
Significant difference	Odds 19: 1 Odds 99: 1	6.4	0.43 0.58	0.14 0.19	0.14 0.19	0.034 0.045	0.011 0.015	

a Dried to constant weight at 70° C.

b Difference not significant.

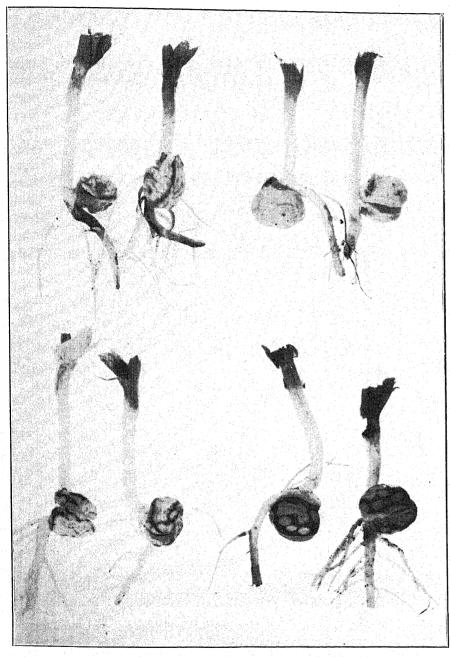


Fig. 3. Effect of seed protectants upon cotyledons of Laxton's Progress garden peas in pasteurized soil. Upper left—nontreated; Upper right—Spergon; Lower left—Semesan; Lower right—Yellow Cuprocide. The dark necrotic areas on the cotyledons in the lower row correspond to the ridges of the wrinkled peas. Spergon did not injure cotyledons, Semesan caused slight necrosis and reduced the rate of cotyledon absorption, while Yellow Cuprocide caused pronounced necrosis, delayed absorption, and reduced seedling growth.

were compared with nontreated seeds in 5 replications each containing 30 seeds. Conditions favored rapid germination and the results in table 4 show that neither of the treatments affected the emergence percentage, the emergence rate, or the weight of seedlings after 3 weeks of growth.

The second trial in pasteurized soil consisted of five seed treatments and a control each replicated 10 times with 15 seeds per row. The seeds were disinfected with mercuric chloride before treatment. Emergence was at about the same rate with all treatments except Yellow Cuprocide with which there was a significantly longer emergence period. It was also the only treatment for which there was a significantly lower seedling weight either with or without cotyledons. The dry weights of seedlings without cotyledons show that seeds treated with Yellow Cuprocide or Semesan produced smaller plants than seeds with other treatments or the control. At least part of this difference, however, is explained by the greater cotyledon weights for the same two treatments (Table 4, column 7).

The cotyledons of plants from the control or those treated with Spergon. Arasan, or New Improved Ceresan were shriveled and much reduced in size within 3 to 4 weeks after planting in the greenhouse. The cotyledons from plants in the Yellow Cuprocide and Semesan treatments were at the same time not shriveled and only slightly absorbed. Particularly upon the Yellow Cuprocide-treated seeds a very pronounced mottled reddish-brown pattern was evident (Fig. 3), this pattern apparently coinciding with the ridges on the wrinkled seed. The sunken areas of the dry seed are less heavily coated with the dust and remain green after the seed swells during germination. The darkened pattern, as shown by microscopic examination, is composed of necrotic cells on the surface of the cotyledons and results from the heavy dosage of the dusts on these areas. With Semesan-treated seed the cotyledon necrosis is mild and apparently has not reduced the total seedling weight or adversely affected subsequent growth. With Yellow Cuprocide, however, the seedling weights were significantly less than with other treatments and as shown in table 2 the yield of seed per plant was reduced. This reduction may have been due to retarded emergence or to the reduced rate of cotyledon absorption or to a combination of both factors.

DISCUSSION

Pea seed were treated with a number of commercial compounds to determine the effect of these materials under some of the planting conditions in California, their effectiveness as protectants against *Pythium ultimum* and *Rhizoctonia solani*, and their effect on plant growth. In addition to the usual emergence percentage, other measures of the effect of the seed protectants were used in some experiments. A disease index combines the degree of injury to the living seedling, and the incidence of pre- and post-emergence damping-off. This figure was, in general, closely related to the stand counts. In some of the experiments the relative rates of emergence were measured by a mean emergence period.

Most trials with seed protectants have been in field soils without knowledge of the specific organisms involved. Because some fungicides are more or less specific in their toxicity relations it is important to measure their protective values in soils infested with different pathogens.

In three greenhouse tests in soil infested with *Pythium ultimum*, the highest protective values were given by Semesan and Yellow Cuprocide; but Spergon, New Improved Ceresan, and Arasan were only slightly less effective. In a single trial a new material, dichloro-naphthoquinone (604) produced excellent stands in heavily infested soil.

In field plantings during the early spring months *Pythium ultimum* appeared to be the chief cause of seed decay but the infestation was much lighter than in the greenhouse trials. In the field, Spergon appeared to be as effective as Semesan and in some trials had definite advantages over Yellow Cuprocide and New Improved Ceresan. Trials with Arasan and Fermate were too limited to justify definite conclusions.

Two greenhouse tests were conducted in soil artificially infested with *Rhizoctonia solani*. Due perhaps to environmental conditions or to the strain of the fungus, infection was limited to post-emergence rot of cotyledons and epicotyls. None of the seed treatments effectively controlled this phase of infection.

The rate of emergence as measured by the mean emergence period or by the coefficient of velocity of emergnce was adversely affected by certain seed treatments. Under warm temperatures, favorable for rapid germination, none of the treatments significantly altered the mean emergence period; but whenever the germination period was prolonged by low temperatures Yellow Cuprocide caused a significant delay in emergence and a reduction in subsequent growth.

In one field experiment planted in January, the emergence of two varieties, Laxton's Progress and Wisconsin Early Sweet, was delayed 5.4 days and 2.8 days respectively by Yellow Cuprocide. A prolonged injurious effect was reflected by a significant decrease in total yield, and yield per plant of dried seed at the end of the season. In two greenhouse experiments, one in sterile soil and one in *Rhizoctonia*-infested soil where temperatures were sufficiently cool, emergence was delayed; and seedling growth in the sterile soil was definitely reduced.

There was some indication of injury by New Improved Ceresan, although the evidence was not so clear as with Yellow Cuprocide. In a field planting of Wisconsin Early Sweet, treatment with New Improved Ceresan caused a definite delay in emergence. In two of three field tests, stands from seed treated with New Improved Ceresan were significantly less than for one or more other treatments. It is not clear, however, whether this was due to injury or to a lower degree of protection. No evidence of injury from the use of Spergon or Arasan was observed in any of the trials, and Semesan produced only slight cotyledon necrosis and some delay of cotyledon absorption without significantly affecting seedling growth or yield of mature plants.

Because other workers have reported evidence of stimulation from treating pea seeds with Spergon this point was tested both in greenhouse plantings in pasteurized soil and in field plantings. In pasteurized soils the results were influenced only by injurious or stimulatory effects since protective effects did not occur. In these experiments there was no evidence that any of the materials tested significantly increased the rate of emergence or the green or dry weight of seedlings.

In field trials both the rate of emergence and yield may be influenced by infection and by relative protective effects of the fungicides, as well as by injurious or stimulatory effects. It is evident from three field trials that none of the treatments hastened emergence as compared with nontreated seeds or with other noninjurious treatments. Yield data from three field plantings with different varieties show no significant differences between Semesan and Spergon. Yellow Cuprocide and New Improved Ceresan, however, resulted in significantly lower yields in two of the three tests. In a single test with a low incidence of infection there was no significant difference in the emergence rate or yield of peas grown from seed treated with Arasan, Fermate, or Spergon although each treatment improved both stand and yield of vine and pods as compared with the nontreated control.

The writers conclude, therefore, that under the conditions of these tests the benefits of seed treatment were due entirely to disease prevention. In the absence of infection none of the seed treatments tested produced more rapid germination or higher yields than did the nontreated control. In the presence of pathogenic organisms no treatment produced significantly higher yields than other treatments that were equally protective and equally non-injurious.

SUMMARY

These investigations comprise a study of the protective values of several commercial seed treatment preparations and their effect on rate of emergence, growth, and yield of garden peas.

A comparison of protective values shows that in soil artificially infested with *Pythium ultimum*, Semesan and Yellow Cuprocide appeared to provide better protection against infection than Arasan, New Improved Ceresan, or Spergon although the differences were not great and all five materials provided stands far superior to the nontreated control. In a single trial dichloro-naphthoquinone also appeared to be highly effective as a protectant against *Pythium ultimum*.

In similar trials in pasteurized soil artificially infested with a strain of *Rhizoctonia solani* that produces damping-off on sugar beets and beans, no seed decay or pre-emergence damping-off occurred upon peas. None of the seed treatments effectively controlled post-emergence epicotyl infection or cotyledon decay.

In field trials where infestations, principally *Pythium*, were light or of moderate intensity all of the chemicals provided adequate protection. Chiefly because of freedom from injurious effects, however, Spergon and

Semesan produced better results in some tests than Yellow Cuprocide or New Improved Ceresan.

Injurious effects, represented by reduced rates of emergence and seedling growth, were caused by Yellow Cuprocide when the seed was planted at low temperature. At moderate or high temperature when germination was rapid no injury by Yellow Cuprocide was apparent. New Improved Ceresan also appeared to delay emergence in one test but it is difficult to determine whether the reduced yield, as compared to those with Spergon or Semesan, resulted from mild injury or from a lower protective value.

Localized necrosis of cotyledons resulted from treatment of Laxton's Progress peas with Yellow Cuprocide and absorption from the cotyledons by the growing plant was delayed. Semesan also produced a mild form of localized cotyledon necrosis and delayed absorption but did not appear to reduce seedling growth or yield of mature plants.

No evidence of chemical stimulation as distinguished from fungicidal protection was observed in these tests.

CALIFORNIA AGRICULTURAL EXPERIMENT STATION. DAVIS, CALIFORNIA.

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THE EFFECT OF MANURE AND OF COMMERCIAL FERTILIZER ON SUSCEPTIBILITY OF YOUNG APPLE TREES TO BLACK ROOT ROT

J. S. COOLEY1

(Accepted for publication November 7, 1944)

There is evidence² that certain conditions that presumably affect adversely the synthesis and translocation of organic materials and other life processes also affect susceptibility of apple trees to black root rot (*Xyluria mali* Fromme). It seemed desirable to get information on the effect of good nutrition versus poor nutrition on the susceptibility of apple trees to this disease; also to endeavor to determine to what extent the usual fertilizer elements influence susceptibility of the host.

EFFECT OF MANURE

In the spring of 1934 Wealthy and Early Ripe trees were set in two nursery plots at the U. S. Horticultural Station at Beltsville, Maryland, preparatory to manuring and inoculating with Xylaria. One plot received manure at the rate of about 14 tons per acre and the other received none. The manure was applied every year for 8 years except in 1938 and 1942. Cultivation continued until 1938 when it was discontinued on account of increased size of trees. Trees were inoculated first in 1936 and then each year through 1942, usually about the middle of July. The inoculations were made one year on one side and the next year on the opposite side of the main root. The method of recording size of lesion was the same as that used in a former publication.³ In taking the record of percentage of infection and size of lesion the shallow lesions that involved only the cortex and usually persisted for a single year were recorded separately from the deep lesions that involved and killed the cambium. Since the percentage of cortical lesions usually corresponded to that of deep lesions, only the latter are presented in this discussion.

By the end of the first year or two the manured trees showed high vigor as evidenced by the large dark green leaves and by the twig elongation. However, there was no visual evidence of over-feeding. On the other hand the nonmanured trees, early in the experiment, began to show evidences of lack of vigor. By the end of the experiment these trees had less diameter and height than those in the manured plot. The general effect, however, in the nonmanured trees was that of low vigor rather than of a starved condition

² Cooley, J. S. Some host-parasite relations of black root rot of apple trees. Jour. Agr. Res. [U.S.] 69: 449-458. 1944.

³ Cooley, J. S. Factors affecting distribution and severity of black root rot of apple trees. Jour. Agr. Res. [U.S.] 65: 299-311. 1942.

¹ Senior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Bureau of Plant Industry Station, Beltsville, Maryland.

The percentage of infection obtained in these 7 years of inoculations varied from year to year, it being usually between 60 and 80 per cent. The percentage of infections obtained in 1937 was lower on these as well as on other plots than that obtained in other years. The number of infections obtained in 1938 was also abnormally low. In these two years the percentage of infection was less on the manured than on the nonmanured plot. In 1937 the deep infections were only 9 per cent on the manured plot and 37 per cent on the nonmanured. In 1938 the manured plot showed 36 per cent infection and the nonmanured plot 57 per cent. However, in 1936, 1939, 1940, 1941, and 1942 the difference between the manured and nonmanured plots was negligible. The general average for the 7 years was 51 per cent infection on manured trees and 61 per cent on the nonmanured trees.

The yearly average diameter of lesion ranged from 18 to 40 mm. There was no striking difference in size of the lesion between the manured and non-manured plots. The average size of lesion for all 7 years' inoculations on trees in the manured plot was 28 mm. and on trees in the nonmanured plot was 26 mm. In the main the infection percentage and the size of the lesions on the trees in this experiment were comparable to those on other experimental plots. In general, the data for seven years of consecutive inoculation show that manuring did not affect either favorably or unfavorably the incidence of the disease. No test has been made of the advance and persistence of the disease on bearing trees of high vigor compared with those of low vigor.

EFFECT OF COMMERCIAL FERTILIZER ON RESISTANCE OF APPLE TREES TO BLACK ROOT ROT

The trees used in this experiment were planted 12 to 20 inches apart in rows four feet apart. The experiment was begun after the trees had been planted three years and when they were beginning to show apparent nutrient deficiency. The seven fertilization combinations consisted of N-K-P, N-K, N-P, K-P, N, K, P, and check, each of which was replicated four times in a randomized arrangement. All fertilizers were applied in March or early in April. Each plot contained 12 linear feet of nursery row in which there were 9 to 12 trees, and the treatments were replicated 4 times, making a total of about 40 trees for each fertilizer treatment. The plots were arranged in four rows with buffer rows alternating with the 4 fertilized rows. The potassium, as muriate of potash, was applied in holes about 8 inches deep and 12 inches apart, 10 inches from each side of the tree row. Nitrogen, as Chilean nitrate of soda, and phosphorus, as 16 per cent acid phosphate, were applied on the surface. Assuming that the fertilized trees fed in the fertilized plot only and that the trees in the buffer rows did not appropriate fertilizers intended for experimental rows, the rate of application was 107 pounds of muriate of potash, 107 pounds of 16 per cent acid phosphate, and 324 pounds of nitrate of soda per acre. The only evidence of fertilizer injury was a yellowing of the foliage and slight defoliation on the plots receiving potash alone.

By the end of the experiment no outstanding differences could be noted in the different plots. Twig elongation and color and size of the leaves did not indicate outstanding differences in nutrient levels. The trees in the check plots did not make quite as much twig elongation as those in some of the nitrogen plots, but they were not in a starved condition.

The percentage of deep infection on the check plot for most years was comparable with the results of other inoculation experiments. In none of the fertilizer treatments did the percentage of infected trees differ greatly from that in the check plot.

During the 4 years the inoculations were made the infection percentage varied somewhat from year to year. In the 3 years 1939 to 1941 inclusive, it usually ranged around 55 to 80 for the various fertilizer treatments. In 1942, however, it was consistently low on all fertilized plots, ranging from about 20 to 30 with an average of 26, while the unfertilized check was 32. The average percentages of deep infections for the 4 years from 1939 to 1942, inclusive, were: check, 62; N-K-P, 62; N-P, 60; N, 61; K, 46; K-P, 67; P, 48.

The average percentages of infection on the plots receiving only potassium and on those receiving only phosphate were low. However, the plot receiving a combination of these two elements had as many lesions as the other fertilized plots or the check. The data indicate that there is no appreciable difference in the percentage of infection resulting from the fertilizer treatments used.

The average size of lesion in mm. for each fertilizer treatment was as follows: check, 26; N-K-P, 24; N-P, 28; N-K, 27; N, 27; K, 26; K-P, 25; P, 24. There was thus no appreciable difference in the size of lesion on the plots receiving the different fertilizer treatments.

PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND.

PULLULARIA PULLULANS STORAGE FRUIT SPOT OF TOMATO

CARLTON F. TAYLOR AND LELAND SHANOR

(Accepted for publication November 28, 1944)

Early in December, 1943, the senior author's attention was called to a spot of tomatoes that was developing on ripe fruit of the variety Break O'Day which had been in cold storage since the latter part of October at West Virginia University, Morgantown. Since the spot was one which was not familiar, isolations were made from fruits at Morgantown. Later a spotted fruit was left at the Beltsville, Maryland, laboratory of the Emergency Plant Disease Prevention Project for additional isolations. Pullularia pullulans (DeBy.) Berkhout (= Dematium pullulans DeBy.) was obtained independently in pure culture in both laboratories. Because this organism apparently has not been reported as a pathogen on tomato fruit, the spot caused by it is being illustrated and described briefly, together with a short account of experimental evidence demonstrating its pathogenicity.

The tomatoes on which the spots later developed were picked on October 6, following a light frost, and were placed on newspaper on a floor to ripen. As fruits ripened they were individually wrapped in oil-treated wrappers and placed in the cold storage room where the temperature was between 1° and 4.5° C. and where the humidity was high. Some of the wrappers had been used previously to wrap sweet potatoes, while others were being used for the first time. The fact that some wrappers had been used and others had not, did not influence the prevalence of the fruit spots. Spots were as abundant on fruit wrapped in unused wrappers as on fruit wrapped in wrappers that had been used before. By late November many of the fruits had characteristic spots and by the end of December practically all of the fruits were affected.

In the early stages of development spots caused by *Pullularia pullulans* appear superficially to be somewhat similar to early anthracnose lesions. At first, the mycelium forms a light colored mat under the epidermis, and the area immediately surrounding it appears water soaked (Fig. 1, a). A depression soon develops and eventually the mycelium in the center becomes deeply pigmented so that the center of the spot is black surrounded by a lighter region. This in turn is surrounded by a water soaked area (Fig. 1, b). Lesions are circular and seldom attain a diameter greater than 1.5 cm. Eventually the epidermis over infected areas cracks and the mycelium develops outwardly to form a relatively solid ridge of fungal tissue, at first light colored, but later becoming black and shining.

When the skin of fruit is peeled away from the flesh over lesions, the underlying fungal growth tends to remain attached to the epidermis in a somewhat hemispherical mass. Pure cultures were isolated easily from these fungal masses. Cultures of isolates grow readily on such standard culture media as potato-dextrose agar and corn-meal agar and produce conidia abun-

dantly at the surface of the culture media. Such colonies are smooth, shining, at first white to cream, later buff or tan, and in surface growth appear distinctly yeast-like. In older cultures, portions of the mycelium within the agar and scattered segments in the surface growth turn black. Such mycelium has rounded cells with much thickened and pigmented walls.

Conidia commonly sprout from any hyaline cell of the mycelium and frequently produce secondary conidia either while attached or upon becoming separated from the parent cell. Sprouting from secondary conidia and the conidia that continue to be formed in this manner often results in the formation of conidial chains. In order to demonstrate the pathogenicity of isolates and the ability of spores to penetrate the epidermis and develop

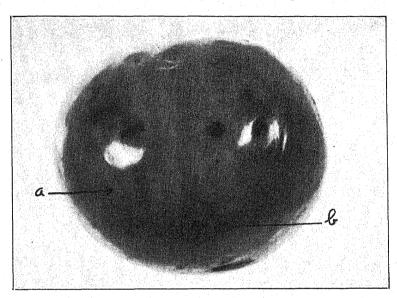


Fig. 1. Tomato fruit of variety Marglobe, showing fruit storage spot produced by *Pullularia pullulans*. a. Spot before pigmentation of the organism has developed. b. Later spot showing central area occupied by darkly pigmented mycelium surrounded by an area of lighter mycelium and an outer water-soaked region. ×about 1.7. (Photograph by W. J. Mead.)

under storage conditions, fruit of the variety Marglobe, after being washed in sterile distilled water, were dipped in a spore suspension, placed in moist chambers, and then allowed to remain in a refrigerator at 7.5° to 10° C. Fruit in varying degrees of maturity from green to ripe was inoculated. After about ten days in the refrigerator typical early lesions were evident on inoculated ripe and nearly ripe fruit. Three or four days later the typical darkly pigmented mycelium became evident in the center of many of these spots. The fruit illustrated in figure 1 was inoculated by being dipped in a spore suspension. Spots developed more slowly on green tomatoes than on ripe or nearly ripe fruit. The fungus developing under the epidermis of green fruit became pigmented almost from the time spots were recognizable. Pure cultures of *Pullularia pullulans* were reisolated from infected

fruit, thus demonstrating beyond a doubt that this fungus was responsible for the fruit spot and may be parasitic to tomatoes under the storage conditions noted.

The temperature of the room in which the spot was originally detected was below that at which tomato fruits are commonly stored and the temperature of the refrigerator used for storage of the inoculated fruit was also slightly below that generally recommended for commercial practice. Whether the parasitic tendencies of this organism for tomato are limited to low temperature environments alone has not been investigated.

BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING, BELTSVILLE, MARYLAND.

DESIGN FOR CONSTANT-TEMPERATURE TANKS

W. A. CAMPBELL AND JOHN T. PRESLEY

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In the course of investigations on guayule diseases it was deemed desirable to study the relation of soil temperatures to infection by soil fungi. For this purpose constant-temperature tanks were built. These were patterned after those developed at the University of Wisconsin, but their construction had to be greatly modified because of the unavailability of preferred materials. These modified constant-temperature tanks, especially those cooled directly by mechanical refrigeration, proved so satisfactory that plans of their construction are herein presented.

The series consisted of six redwood tanks each divided into two compartments. Four of the tanks (8 compartments) were electrically heated and thermostatically controlled to provide temperatures above that of the water supply (approximately 70° F.). Four compartments in two tanks were cooled by individual refrigeration coils to provide temperatures below 70° F. The temperatures in both the electrically heated and the mechanically cooled tanks did not fluctuate more than 2 degrees from the desired temperature.

Each compartment contained 8 metal cans, 8 inches in diameter and 18 inches deep, in which plants were grown directly, or in 6-inch unglazed pots embedded in sand to facilitate aeration and drainage. Details of the construction of the redwood tanks and the arrangement of the cans are in figure 1. Blueprints of the tanks may be obtained from the Guayule Research Project.³

Construction of the Tanks.—The tanks were of 2-inch planed redwood planks splined with $\frac{1}{2} \times 1$ -inch redwood strips and firmly bolted together by long iron rods. In order to replenish water lost by evaporation, as well as to provide a means of quickly changing the temperature within a tank, a $\frac{1}{2}$ -inch water line with valve was connected to the bottom and an overflow pipe arranged at the top.

Electrically Heated Unit.—Each individual compartment in the four tanks designed for operation at temperatures above that of the water supply was electrically heated by 30 feet of lead-covered heating cable connected to a 110-volt line. The capacity of the heating cable was sufficient to furnish a temperature range from 70° to 110° F. The desired temperature within each compartment was maintained by an adjustable thermostat connected to each heating unit.

Mechanically Cooled Unit.—An ordinary refrigerating unit (Kelvinator

¹ Jones, L. R., J. Johnson and J. A. Dickson. Wisconsin studies upon the relation of soil temperature to plant disease. Wisc. Agr. Exp. Stat. Res. Bul. 71. 1926.

² The details of the construction of the tanks, as well as the arrangements for heating and cooling, were developed by M. G. Still, Chief Foreman, Construction and Maintenance, Forest Service. Emergency Rubber Project.

Forest Service, Emergency Rubber Project.

3 The drawings of the constant temperature tanks were prepared by W. E. Deemer, Associate Mechanical Engineer, Forest Service, Emergency Rubber Project.

series SSB, powered by a ¹/₃-horsepower electric motor) furnished refrigeration for the 4 compartments in which temperatures below 70° F. were main-

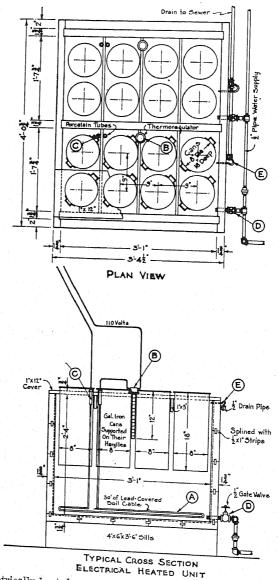


FIG. 1. Electrically heated constant-temperature tanks. Upper diagram: Plan view giving dimensions of redwood box and arrangement of galvanized cans. Lower diagram: lation of relays not illustrated.

A. 30 feet of lead-covered soil cable, 110-115 volt, 700 to 750 watts capacity. B. Thermostat (American Instrument Co., "Quickset" bimetal thermoregulator). C. Porcelaintube insulators. D. Water intake at bottom. E. Overflow pipe of 4-inch copper tubing emptying into 4-inch galvanized drain.

tained. This unit had sufficient capacity to maintain 2 compartments at 40° F. and 2 at higher temperatures, but would not maintain all 4 at 40° F.

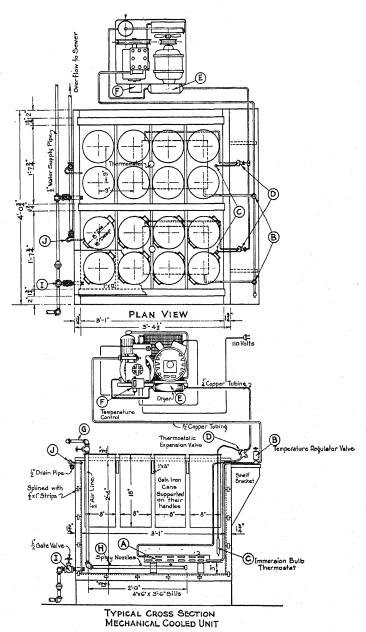


Fig. 2. Mechanically cooled constant-temperature tanks. Upper diagram: Plan view showing arrangement of tubing connecting two of the cooling coils with the refrigeration unit. The installation now in use has 4 compartments connected to the same refrigerating unit. Lower diagram: Cross-sectional view, showing details as to the arrangement of the installations in a single compartment.

A. Cooling coil. B. Temperature-regulator valve. C. Immersion-bulb thermostat. D. Thermostatic expansion valve. E. Dryer. F. Temperature control. G. Air line. H. Spray nozzles to provide agitation. I. Water intake at bottom. J. Overflow pipe (½-inch conner tubing)

copper tubing).

Installations at the refrigeration unit consisted of a dryer (E) and a temperature control unit (F) connected as shown in figure 2. The cooling coils (A), one to each compartment, were connected to the refrigerating unit. Each cooling coil was supplied with a temperature-regulator valve (B) and an immersion-bulb thermostat (C) operating in conjunction with a thermostatic expansion valve (D). Different temperatures could be maintained in each of the 4 compartments by adjusting each temperature-regulator valve. In actual practice temperatures from 40° to 65° F. were commonly maintained.

In order to maintain uniform temperatures within the tank, an air line (G) with 3 spray nozzles (H) was placed in the bottom of each compartment to provide agitation and circulation of the water.

SPECIAL GUAYULE RESEARCH PROJECT,

BUREAU PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING, SALINAS, CALIFORNIA.

PHYTOPATHOLOGICAL NOTES

Poria microspora in House Timbers.—The urgent demands for wood during the war period have often resulted in the use of poorly seasoned lumber which, when shipped long distances in carload lots, sometimes arrives at retail yards with mats of fungus mycelium covering many of the timbers. This lumber is often sold to building contractors before much more air drying can take place, even in those yards where open piling is employed. The use of such partially dried and contaminated lumber in war-time housing construction, coupled with restricted ventilation within the walls of houses insulated by modern methods, creates moisture conditions that favor the growth of wood-decay fungi. Under such conditions a native but newly described fungus, Poria microspora Overholts, has been found causing serious decay in a new house of modern construction at Syracuse, New York.

The fungus apparently was present in the lumber at the time of construction. Although the house was heated and ventilated immediately after plastering, the moisture conditions within the insulated walls were such as to permit decay. It is believed that moisture from the drying plaster raised the relative humidity of the air within the wall, and the uprights, sheathing, and inner surface of the siding absorbed sufficient moisture, either directly from the air or as a result of condensation, to permit active decay.

No sporophores were formed on the decaying wall timbers but when several boards of the decaying pine sheathing were placed in the decay cellars at the New York State College of Forestry fruit bodies were produced on the wood in approximately one year. A thick, light-colored mycelial mat appeared first and large drops of colored liquid were profusely formed upon its surface. Tubes formed soon afterwards. The determination of *Poria microspora* was made by the junior author and confirmed by Dr. L. O. Overholts of the Pennsylvania State College, and a further confirmation was made by means of cultural as well as morphological characters by Dr. Mildred K. Nobles, Junior Plant Pathologist, Division of Botany and Plant Pathology, Central Experimental Farm, Ottawa, Canada.

Poria microspora appears to form fruit bodies very rarely, as these have been reported from only three other widely scattered stations. It was first found on Sitka spruce in the Queen Charlotte Islands off the coast of British Columbia, and later on flooring at Sharon, Wisconsin, and on a southern pine beam in a boat at Mystic, Connecticut. Its existence in Eastern Canada at Calumet, P. Q., has been determined from cultures of spores caught in spore traps. Infected wood has been demonstrated over a still greater area, by cultures of Sitka spruce and Douglas fir imported into Great Britain from Canada, and from Douglas fir in Oregon and Toronto. So far it has been found in British Columbia only on standing trees.² This extensive but sporadic distribution probably indicates that the fungus is widespread but has

Nobles, Mildred K. A contribution toward a clarification of the Trametes serialis complex. Canad. Jour. Res. (C) 21: 211-234. 1943.
 Nobles, M. K. Op. cit.

been overlooked because of the rare appearance of the fruiting structure; perhaps also because the plant has been confused with *Trametes serialis* Fries.

Externally *Poria microspora* (Fig. 1) closely resembles the resupinate and more familiar *Trametes serialis*. The fruiting body, formed in the decay cellars, is dull white, about 3 mm. thick, and the texture when fresh was dry and soft-papery, quite different from the much tougher *T. serialis*. The

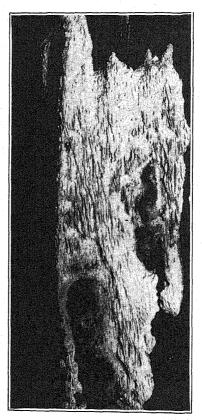


Fig. 1. A fruit body of Poria microspora produced on southern pine sheathing.

tubes reached a length of 8 mm. on a sloping surface, and the openings or pores averaged about 2–2.5 per mm. The subiculum hyphae bear abundant clamps. The spores are smooth, hyaline, oblong to oblong-ellipsoid, 5–6 \times 2.5–3 μ . These microscopic characters serve to separate *P. microspora* from *T. serialis*, which lacks clamp connections on the subiculum hyphae and also has larger spores, 7–9 μ long.—Ray R. Hirt and Josiah L. Lowe, New York State College of Forestry, Syracuse, New York.

The Name of Ansatospora macrospora.—In the summary of his recent paper on a storage rot of celery, Newhall¹ states: "The fungus causing this ¹ Newhall, A. G. A serious storage rot of celery caused by the fungus Ansatospora macrospora n. gen. Phytopath. 34: 92–105. 1944.

disease is thought to be the one previously described by Osterwalder as the cause of a pansy leaf spot, named Cercospora macrospora by him. Its identity with the fungus causing anthracnose of caraway, described and named Cercospora cari by Westerdijk and van Luijk, also is established. Its name is changed to Ansatospora macrospora (Ostw.) n. gen. chiefly on the basis of the long prominent appendage protruding from the basal cell of the conidium."

The purpose of this note is to point out that in 1880 Robert Hartig² described what is undoubtedly the same fungus as causing a destructive disease of maple seedlings. He called this organism Cercospora acerina. Later, Frank³ made the statement that Hartig had determined the fungus wrongly and without further comments he named it Sporidesmium acerina (Hart.) Frank. It is presumed that Frank examined some very old material in which the conidia are quite dark and frequently swollen. Still later Arnaud observed the fungus on maple seedlings in France but evidently only in the young, hyaline condition, for he changed the name to Cercosporella acerina (Hart.) Arn. Like Osterwalder, Hartig failed to mention or recognize the conidial appendages as such, though these structures are clearly shown in the illustrations of both authors. That Hartig actually saw the appendages is clear from his drawings and from his comments. He states that if the slender tip of a conidium is broken before growth is completed, then a substitute tip grows out somewhere near the end. If this substitute tip occurs on the slender part of the conidium, it grows out at a right angle, and if it occurs lower down on the broken conidium then it grows out "bayonet like." In his own words: "Bricht vor Beendingung des Wachstums die zarte Spitze ab, dann wächst nahe dem Ende seitlich eine Ersatzspitze hervor, die dann, wenn sie an dem zarten Theile der Conidie sich bildet, rechtwinklig, Fig. 5a, wenn sie an einer tiefer unten abgebrochenen Conidie entsteht, bajonettartig hervortritt Fig. 5b." He evidently misinterpreted the appendages for outgrowths in response to injuries. Hartig gave no spore measurements other than those indicated by the stated magnifications on his drawings, but the dimensions recorded by Saccardo,6 $120-150 \times 7-8 \,\mu$, fall well within the range observed by us and other workers.

Hartig's drawings, particularly where he illustrates typical conidia, an appendage, and the characteristic dark, thick-walled resting mycelium, and his description of the disease on cotyledons and other parts of maple seedlings, resulting in their death, indicate that his fungus is the same one considered by the following workers: Arnaud, Frank, Osterwalder, Westerdijk

² Hartig, Robert. Der Ahornkeimlingspilz, *Cercospora acerina* m. Untersuch. Forstbot. Inst. München 1: 58-61. 1880.

 ³ Frank, A. B. Krankheiten der Pflanzen. Band 2. 576 pp. Breslau. 1896.
 ⁴ Arnaud, G. Le mildou des lilas et la maladie des cotyledons d'ērable. Bull. Soc. Path. Veg. France 5: 58-60. 1918.

⁵ Osterwalder, A. Ueber die durch *Cercospora macrospora* Osterwalder verursachte Blattkrankheit bei den Pensees. Mitteil. der Thurg. Naturf. Gesells., Heft 25: 59–80.

⁶ Saccardo, P. A. Sylloge Fungorum 4: 465. 1886.

and van Luijk,7 Newhall, and ourselves. On the basis of priority, this fungus, with a list of known synonyms, therefore becomes:

Ansatospora acerina (Hart.) n. comb.

Cercospora acerina Hartig.

Sporidesmium acerina (Hart.) Frank.

Cercosporella acerina (Hart.) Arnaud.

Cercospora macrospora Osterwalder.

Cercospora cari Westerdijk and van Luijk.

Ansatospora macrospora (Ostw.) Newhall.

—H. N. HANSEN and C. M. TOMPKINS.

Division of Plant Pathology, University of California, Berkeley, California.

The Culture Designated Madison 517 Identified as Polyporus tulipiferus. 1—A fungus originally designated by the Forest Products Laboratory at Madison, Wisconsin, as Fomes annosus (Fries) Cooke, and later as Madison 517, has been used extensively in the United States for more than 20 years as one of the standard laboratory test organisms to determine relative toxicity of various wood preservatives, especially creosotes. Cartwright (see Robertson,2 p. 33) was the first to suggest that this fungus was not F. annosus. From a comparison of cultures, and the evidence of a fruit body formed in culture, he stated that the fungus in question was Polyporus tulipiferus (Schw.) Overh. He did not describe the fruit body on which this conclusion was partially based. Cartwright and Findlay³ later stated that it appeared to be identical with a culture of P. tulipiferus obtained from Canada, and Cartwright⁴ in a subsequent publication states, in discussing this fungus, "in due course small fruit bodies were formed on beech blocks which appeared to be those of P. tulipiferus." The fruit bodies were not described. Richards compared Madison 517, P. tulipiferus 691, and typical cultures of F. annosus, and found that the two former had certain morphological and physiological characters in common, and differed in some respects from F. annosus. She considered the identity of Madison 517 to be uncertain.

The writers inoculated sections of cottonwood (Populus deltoides Marsh) and hackberry (Celtis occidentalis L.) with a culture of Madison 517 obtained from Dr. H. Schmitz, University Farm, St. Paul, who obtained his culture from Dr. Richards at the Forest Products Laboratory, and one of Polyporus tulipiferus isolated from a typical sporophore growing on a large

Westerdijk, Johanna, and A. van Luijk. Eine Anthraknose des Kümmels (Carum carvi). Meded. Phytopath. Lab. Willie Commelin Scholten Baarn 8: 51-54. 1924.
 Paper No. 2201, the Scientific Journal Series, Minnesota Agricultural Experiment

² Robertson, W. A. Report of the Director of Forest Products Research for the year

6. Rep. For. Prod. Res. Board, 1935. 1936. 8 Cartwright, K. St. G. and W. P. K. Findlay. Principal decays of softwoods used in Great Britain.

reat Britain. 106 pages. 1938.

4 Cartwright, K. St. G. The relation between field and laboratory work in mycology. Trans. Brit. Myc. Soc. 22: 222-238. 1939.

⁵ Richards, C. Audrey. The doubtful identity of Fungus No. 517. Proc. Amer. Wood-Preservers Assoc. 33: 104-106. 1937.

wound of a living hackberry in St. Paul. The method of inoculation was that described by Darley and Christensen. Two to five months later the inoculated pieces of wood were placed under a greenhouse bench at 85° F. and watered occasionally. When fruit bodies began to appear, water was dripped slowly but continuously on the wood pieces, and within 3 to 4 weeks

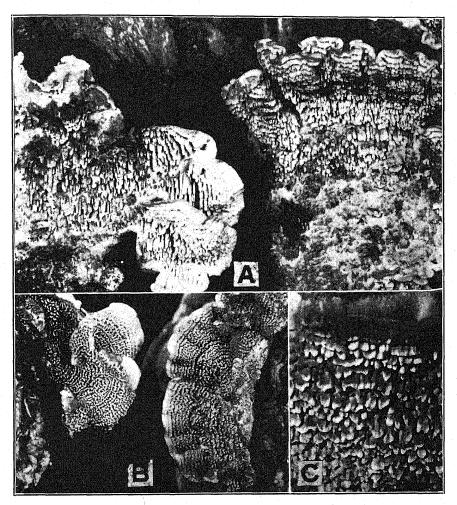


Fig. 1. A and B. Two views of the same fruit bodies. Note concentric arrangement in B. Left, *Polyporus tulipiferus*; right, Madison 517. C. Effuse-reflexed pileus of Madison 517.

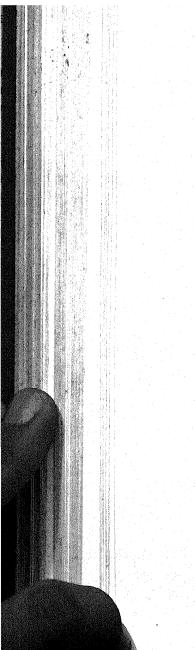
thereafter the fruit bodies attained their full size. The wood was inoculated in August, 1943, and a considerable number of fruit bodies matured in August, 1944.

Macroscopically the fruit bodies of both isolates were typical of those formed by *Polyporus tulipiferus* in nature—resupinate to effused reflexed,

⁶ Darley, Ellis F. and Clyde M. Christensen. An unusual sporophore of *Trametes suaveolens* produced on artificially inoculated wood. Phytopath. 33: 328–330. 1943.

the upper surface white, villose, and faintly zoned, the under surface with irregular, daedaloid pores which soon broke up into concentrically arranged teeth (Fig. 1). Fruit bodies formed by Madison 517 were sent to Dr. L. O. Overholts, Pennsylvania State College, who identified them as $P.\ tulipiferus$.

Spore prints were obtained from fruit bodies of both isolates, and fresh pores and teeth were sectioned free hand, mounted in water, and the microscopic structures measured with the aid of a screw micrometer and an oil immersion lens. The average size of 60 cast spores of Madison 517 was $2.8 \times 5.2~\mu$, with a range of $1.9-3.5 \times 4.1-6.5~\mu$, while the average size of 60 cast spores of *Polyporus tulipiferus* was $2.8 \times 4.9~\mu$ with a range of $2.3-3.3 \times 3.7-6.4~\mu$. Twenty-five basidia from each sporophore averaged $5.2~\mu$ in diameter, the range in Madison 517 being $4.7-5.9~\mu$ and in $P.~tulipiferus~4.7-5.2~\mu$. Cystidia were plentiful in the hymenia of fruit bodies of both isolates, and were typically encrusted, as is described for P.~tulipiferus; the cystidia protruded up to $30~\mu$ beyond the basidia in the fruit bodies of both isolates. The above evidence proves conclusively that what has hitherto been known as Madison 517 is P.~tulipiferus.—Ellis F.~Darley, and Clyde M. Christensen, University Farm, St. Paul, Minnesota.



MOSAIC OF THE COMMON COLEUS

D. B. CREAGER

(Accepted for publication September 15, 1944)

Because of their colorful, variegated, and beautifully shaped leaves, numerous horticultural varieties of *Coleus Blumei* Benth. are used as ornamental plants. Coleus is used extensively as a bedding plant, especially in parks, cemeteries, and home flower beds, and frequently as a house plant. Coleus is not of major importance as a commercial florists' crop, but it does occupy an important place as a minor crop in the florists' industry.

Since it is the shape and colorful pattern of the leaf which make Coleus plants valuable as ornamentals, any trouble that mars the color quality, color pattern, or shape of the leaf seriously affects their decorative and economic value. Such a trouble was found in several varieties of Coleus growing in the Natural History Survey greenhouses in Urbana, Illinois, during the winter of 1941. Affected plants were off color, faded, mottled, and splotched, and the general brilliance and color effect of the planting was altered (Fig. 1, A).

Plants in which this trouble was first discovered had been obtained as cuttings from another greenhouse, and upon examining the source plants the same disease was found. Since its discovery, the malady has been observed in a number of widely separated commercial greenhouses and outside plantings. Since the Coleus is propagated vegetatively by cuttings and has been so extensively shipped, probably the disease is widespread.

At the outset, the symptoms and general nature of this Coleus trouble were suggestive of those caused by virus infection in other plants, yet no such disease of Coleus seems to have been reported. No reference to a virus disease of Coleus appears in the relatively recent compendiums on plant viruses by Smith (7), Holmes (5), Bawden (1), and Cook (2, 3). Price (6) lists Coleus Blumei Benth. as being susceptible, upon artificial inoculation, to viruses causing tobacco necrosis, tobacco ringspot, tomato ringspot, cucumber mosaic, and alfalfa mosaic, and Hildebrand (4) reports the plant susceptible to the tomato-ringspot virus. Both Price and Hildebrand considered the host susceptible if the virus could be recovered from inoculated plants, whether or not symptoms of infection occurred. Neither of these workers described the appearance of their Coleus plants following inoculation.

In reference to the possible occurrence of a virus disease in *Coleus Blumei*, Cook (2) stated, "It has been suggested that some of the variegations in this and other species may be due to a virus, but there is no proof." In contrast to the sense of Cook's statement, the effect of the Coleus trouble considered here is to alter or destroy established color patterns rather than to create them.

In allusion to the characteristic symptoms of leaf mottling, this disease is being called Coleus mosaic.

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VARIETIES AFFECTED

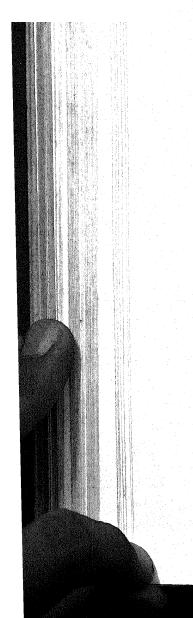
By examination of plants growing in commercial and experimental plantings during the past three years, 40 varieties appear to be susceptible to Coleus mosaic, as indicated by symptoms. Positive symptoms of Coleus mosaic have been found in the following varieties: Beauty, Beckwith Gem, Big Tim, Brilliancy, Centaur, Chicago Bedder, Coral, Daudet Sport, Defiance Sport, Ella Cinders, Filmore Beauty, Firebrand, Firecrest, Fire Flame, Eldorado, Empress of India, Green and White, Glory of Autumn, Gold Bound, Golden Bedder, Helen, Hollywood, Hurricane, Improved Hollywood, Jewel, LaVerne, Leopard, Lord Alverson, Massey, Mme. Caroline Beck, Mrs. Turner, New Defiance, Rainbow, Red Verschaffelti, Riverside, Rob Roy, Salvador, Setting Sun, Verschaffelti, and Yellow Jack. Cross inoculations, using affected plants of Beauty, Centaur, and Gold Bound as sources of inoculum and clones propagated from selected healthy plants of the Gold Bound variety as test plants, demonstrated that all three varieties were affected with the same disease. From observations and results of experimental studies, apparently many commercial varieties of Coleus are susceptible to Coleus mosaic.

SYMPTOMS

In many respects, the symptoms of Coleus mosaic resemble those expressed in other plants affected with virus diseases, but since leaves of most varieties of Coleus present colors, especially shades and tints of red, other than green, color changes in the leaves of Coleus are strikingly different from those seen in the green leaves of most plants. The most striking symptoms of Coleus mosaic are those connected with changes in foliage color.

Since there are so many varieties of Coleus, each with its own color pattern, a description of color changes due to Coleus mosaic cannot be given for all of them. In some varieties the color change symptoms are clearly expressed, but other varieties have leaves so elaborately variegated that these symptoms are quite obscure or masked. Since symptoms involving color changes show quite clearly in the leaves of the Gold Bound variety and since that variety was used in transmission studies described later, this discussion of symptoms of Coleus mosaic will be restricted, for most part, to that variety.

Gold Bound leaves are large and their color pattern is simple (Fig. 1, B). They are deep maroon with a chartreuse green edging above and uniformly willow green below. When affected with mosaic, they lose their brilliance and their uniformly lustrous maroon upper surface becomes mottled or splotched with light or dark maroon, brownish, bronze, greenish, reddish, yellowish, and whitish areas (Fig. 2). Also, the discolored areas often assume such forms as zonal ring spots, oak leaf patterns, and irregular, zigzag, or hieroglyphic markings.



Ring spots can be dark, almost black-red or maroon in the center, surrounded by lighter red or maroon zones, or dull, brownish red in the center, surrounded by one or several light greenish lines or zones. Oak-leaf patterns

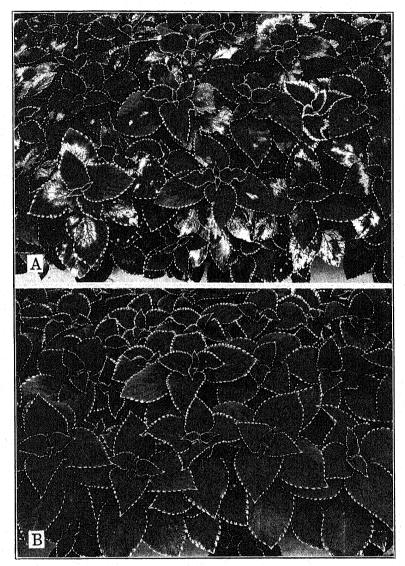


Fig. 1. Coleus mosaic in the Gold Bound variety. A. Mosaicked plants showing typical mass effect of the disease on shape and color pattern of leaves. B. Healthy plants showing typical shape and color pattern of the leaves.

are similar to the ring spots, in that the zigzag area across the leaf is lighter maroon, dull greenish red, brownish green, green, or yellow. Different zones of all gradations of colors can be present in one pattern. Leaves with this pattern are usually lighter maroon, yellowish or dull greenish red towards

the base and black-red or darker maroon than normal just beyond the pattern towards the tip of the leaf. Clearing of veins, especially of the maroon color, is usually present in the oak-leaf pattern, the cleared veins radiating marginward in the midrib and most prominent lateral veins. Vein clearing

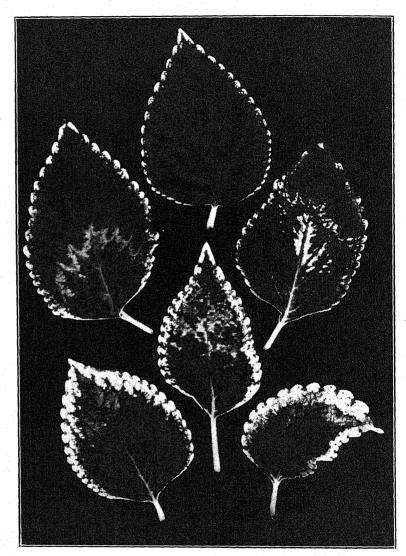


Fig. 2. Effect of Coleus mosaic on Gold Bound leaves. At the top, a normal leaf; below it, five leaves showing oak-leaf patterns, mottling, vein-clearing, fading, and malformation.

also occurs on mottled leaves, although less frequently than on those with the oak-leaf pattern or irregular, stellate ring spots.

Necrotic lesions are not common, but occasionally scattered spots $\frac{1}{8}$ to $\frac{3}{4}$ inch across occur on leaves of affected plants during the winter months and

early spring. These lesions can be simple spots, or they can be circular to stellate, zonal ring spots. The centers of ring spots can be necrotic, or of living green tissue, surrounded by a series of several discontinuous necrotic lines. The color of the necrotic tissue is blackish brown to black.

Affected leaves are often puckered, asymmetric, and rugose (Fig. 2). Puckering and crinkling are often associated with the occurrence of oak-leaf markings, large ring spots, and other strongly marked patterns. Frequently, affected leaves are stunted; occasionally, branches are markedly stunted; and, least often, entire plants are stunted and distorted.

TRANSMISSION STUDIES

Since all known plant viruses can be transmitted by grafting, the graft method was used to determine whether or not Coleus mosaic is caused by a virus. Healthy plants of the Gold Bound variety, all members of the clonal progenies of five healthy individuals, were used as understocks, while shoots taken from diseased plants were used as scions. In both series of grafting tests, the first in January, the second in June, 1942, the cleft graft was used. The stock plants, which averaged approximately 8 inches in height, were prepared for grafting by removing the tops of the plants, 3 to 5 inches below the tips. Then the top of the remaining stem was split down the middle for about an inch. The scion, a shoot taken from a diseased plant with its base trimmed to a wedge, was inserted into the split of the stock and lightly bound in with wrapping cord. The grafted plants were kept in a shaded moist chamber in the greenhouse for about 7 days, until union of scion and stalk was established.

The first series consisted of 28 graft-inoculated plants and a number of comparable check plants to which no scions were grafted. Typical symptoms of Coleus mosaic developed in the Gold Bound foliage of 24 of the grafted plants, but no symptoms appeared in the check plants.

The second series consisted of 75 graft-inoculated plants with an equal number of comparable check plants, the checks being derived by rooting the tops of the plants to which scions were grafted. All 75 grafted plants became infected, producing typical symptoms of Coleus mosaic, and all checks remained healthy.

The first indications of transmission became noticeable approximately 3 weeks after grafting. The earliest symptoms, in most cases, appeared in the young leaves of lateral shoots originating just below the graft union. In many of the grafted plants systemic infection appeared to progress through two stages. These two stages were not observed in all cases, but when they did occur the first stage symptoms consisted of narrow, necrotic lines forming ring spots and other irregular markings in leaves, in the main stem of the stock just below the graft, and in lateral branches of the stock originating near the union. In leaves the lines were very narrow and usually appeared as a series of zigzag parallel lines or ring spots. These lines seemed to have little or no relationship with the vein structure of the leaf,

some crossing the veins at right angles, others crossing them diagonally, and still others paralleling them. These necrotic lines in leaves usually did not develop into the marked necrotic spots which sometimes develop in plants in which systemic infection has been established. In stems, the zigzag lines would sometimes completely surround the stem, but more frequently they would be restricted to one side only, these lesions occurring as single lines or as wave-like series of parallel, parabolic lines. Occasionally, lines in the stem developed into broad necrotic areas, sometimes of such an extent that small branches near the graft union were killed and rather frequently the scion was killed as a result of the complete necrosis occurring at or just below the graft union. Such stem lesions have not been observed in diseased plants found in nature and have not been observed in plants propagated as cuttings from graft-inoculated individuals.

Second-stage symptoms occurred soon after the appearance of the first in the form of characteristic color-fading, mottling, splotching, and formation of oak-leaf patterns and ring spots. Symptoms were, in general, like those in diseased plants from which the scions were originally taken. These second-stage symptoms appeared in all graft-inoculated plants which became infected, but were not always preceded by the first-stage symptoms. After they appeared, symptoms identical with those developed in the first stage did not continue development or recur.

The infective principle has been transmitted not only from the original diseased plants to healthy plants but also from graft-inoculated plants to healthy plants. Further, it has been carried repeatedly from one generation of plants to the next in cuttings taken both from graft-inoculated plants and from originally diseased individuals. The results of these studies clearly indicate that Coleus mosaic is caused by a transmissible virus.

Thus far, all attempts to transmit the Coleus mosaic virus mechanically from diseased to healthy plants have failed. Freshly expressed juice from infected plants, rubbed on leaves of healthy Coleus and Turkish tobacco, with or without the use of carborundum powder, gave no symptoms of infection on either plant. Likewise, when expressed juice was injected with a hypodermic needle into veins, petioles, and young stems of healthy Coleus plants, no evidence of transmission resulted.

Since Coleus mosaic occurs in some plantings but not in others, and since it has been found in many different varieties in those plantings where it occurs, there must be some natural method of virus transmission. When a block of several hundred healthy stock plants and a block of diseased plants were grown side by side in pots on a greenhouse bench, positive indications of transmission appeared. Three plants belonging to two different clonal progenies of healthy plants, adjacent to the block of diseased plants, became infected. Also, at the opposite end of the block of healthy plants, nine other plants representing clonal progenies of four healthy individuals became diseased. This transmission occurred only in the two restricted areas, and only part of the plants representing any one clone became infected. These

observations, coupled with the failure of attempts at mechanical transmission, suggest that insects may be the natural agents of transmission.

SUMMARY

Coleus mosaic, a disease causing mottling, splotches, ring spots, oak-leaf patterns, and hieroglyphic markings on leaves, has been found in a number of greenhouse and outside plantings of Coleus.

Indications are that Coleus mosaic is widespread and that many commercial varieties of Coleus are susceptible.

Grafting tests have clearly proven that Coleus mosaic is caused by a transmissible virus.

Observations made in experimental and commercial plantings indicate that there is some natural means of spread of the Coleus mosaic virus. Since all attempts to transmit the virus mechanically have failed, it is suggested that insects might be vectors.

SECTION OF APPLIED BOTANY AND PLANT PATHOLOGY, ILLINOIS STATE NATURAL HISTORY SURVEY, URBANA, ILLINOIS.

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RHIZOCTONIA NECK ROT OF GLADIOLUS

D. B. CREAGER

(Accepted for publication November 1, 1944)

For several years a neck rot of gladiolus has been under observation and study in the commercial gladiolus-growing area of Kankakee County, Illinois. This disease occurs in scattered plantings being grown from cormels. It destroys the plants in long, continuous sections of the rows (Fig. 1). It generally appears early in May. As the season progresses, the affected sections of the rows grow longer and longer, some of them reaching six to seven feet at the time the corms are ready for harvesting.

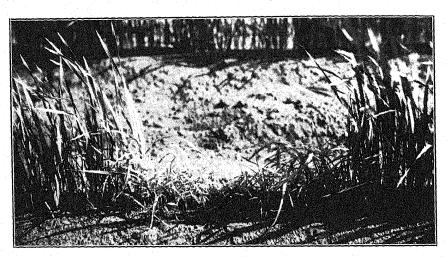


Fig. 1. Gladiolus plants, Opalescent variety, affected with Rhizoctonia neck rot, showing a typical section of a row in which the plants have been killed; natural inoculation.

In the majority of plantings in which the disease has been observed, estimated losses have been less than 25 per cent, but nearly complete destruction of plantings of some varieties has been observed in several instances, especially where several spots of infestation occurred in the rows within a few feet of each other.

Neck rot has appeared in plantings made both on land where gladiolus had not previously been grown and on land where they had been grown for years. Little, if any, difference in the frequency of occurrence of the disease under the two conditions was observed. Severe infection was observed in Rewi Fallu, Picardy, Giant Nymph, Opalescent, Pearl Harbor, Dr. F. E. Bennett, and Flaming Sword varieties. In one planting, the Queen of Bremen variety showed no signs of infection although it was growing alongside other varieties which were severely affected, indicating that it might be resistant to the malady.

Infection occurs in the basal parts of the leaves (Fig. 2, B and C), about one inch below the soil surface, causing lesions which are at first soft and

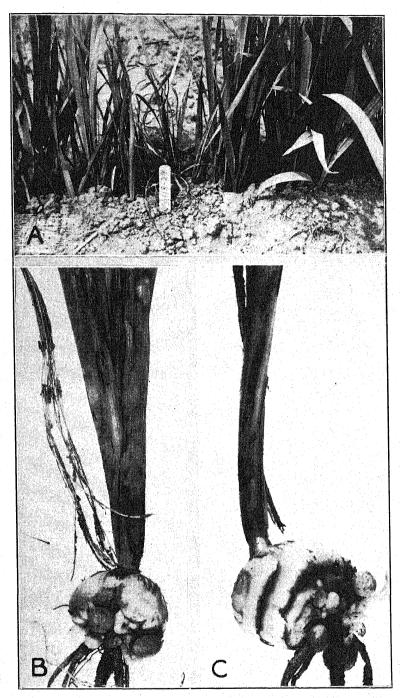


Fig. 2. Gladiolus plants, Picardy variety, affected with Rhizoctonia neck rot; artificial inoculation. A. A short section of a row in which the plants adjacent to the point of inoculation have been killed. B. Necrotic lesions on leaf bases; a typical disintegrated, shredded leaf at left. C. Corm lesions at points of scale attachment, giving the corm a horizontally striped appearance. All inoculated August 3, 1940; photographed October 1, 1940.

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hydrotic, later brown and necrotic. Eventually the bases of infected leaves are shredded, because of disintegration of the succulent parenchyma tissue between the veins. Basal infection of the leaves usually causes wilting and ultimate death of the entire above-ground portions of the plant. However, when large plants become infected, only the outer leaves may be killed or the necrotic lesions may fail to bring about the death of the infected leaves.

Occasionally, leaf sheaths and corm scales on affected plants are destroyed, and long, narrow, brown lesions develop on the corms where the infected scales were attached (Fig. 2, C). These lesions give the corm a horizontally striped appearance. When the top of the plant has been killed, corm development ceases. Consequently, the earlier infection occurs the greater is its effect. At harvest time, one commonly finds no corms, or very small ones, where infection occurred early, but finds progressively larger corms where infection occurred at later times.

If the dead tops are carefully lifted from the soil, a characteristic cobweb-like mycelial growth, to which sand and soil particles cling, is almost always found on them. Its presence aids in diagnosing the trouble, since microscopic examination and cultural studies have shown it to be the mycelium of the pathogen which causes the disease.

A fungus of the genus *Rhizoctonia* has been consistently isolated from fresh lesions on affected leaf bases. Microscopic and cultural comparisons of this fungus with cultures of *Rhizoctonia solani* Kühn isolated from potatoes, poinsettia, and carnations indicate that it is at least a strain of the same species.

Inoculations on plants grown in the greenhouse and on plants in the field have produced typical symptoms of the disease in the Picardy and Rewi Fallu varieties. Pieces of agar medium or barley grains, in which the fungus was growing in pure culture, were placed against the bases of the plants just below the soil surface. The first symptoms of infection appeared within seven days after inoculation, showing on young plants as hydrotic lesions and causing sudden wilting and death of the leaves (Fig. 2, A). On larger, older plants symptoms appeared as large necrotic lesions in the leaves (Fig. 2, B). In these artificial inoculations, the pathogen spread in the row from plant to plant, giving rise to dead sections in general resembling those seen under natural conditions. Typical lesions developed in the scale attachment areas on the corms of many of the infected plants (Fig. 2, C).

The pathogen was readily isolated from the inoculated plants, thus completing good evidence that neck rot of gladiolus is caused by a strain of *Rhizoctonia solani* Kühn.

Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana, Illinois.

SELENOPHOMA BROMIGENA LEAF SPOT ON BROMUS INERMIS'

J. LEWIS ALLISON

(Accepted for publication November 17, 1944)

INTRODUCTION

Smooth bromegrass, *Bromus inermis* Leyss., has become an important forage and pasture grass in the North Central States since 1930. This paper presents work on a leaf spot disease destructive to the foliage of *B. inermis* in nurseries at University Farm, St. Paul, Minnesota. Studies pertinent to the cultural characteristics of the causal organism have been reported by the writer (1, 2).

THE DISEASE

History, Distribution and Description

The causal organism was identified as Septoria bromigena Sacc. (9). It was first described and named from diseased plants collected by Brenckle (4) in North Dakota in 1918. In studies on Septoria spp. on Gramineae in the Pacific Northwest Sprague and Johnson (10) have determined that species with nonseptate, falcate spores, borne in small globose pycnidia, with coarse globose peridial cells, are more logically assigned to Selenophoma Maire than to Septoria Fries. Accordingly, Septoria bromigena becomes Selenophoma bromigena (Sacc.) Sprague and Johnson.

Selenophoma was collected in many localities in Minnesota, Wisconsin, North and South Dakota, and Manitoba. The disease has been reported by Fischer et al. (8) as "almost universal east of the Rockies except in the drier areas."

Symptoms produced by Selenophoma on Bromus inermis are very similar to those caused by species of Septoria attacking other gramineous hosts. Soon after growth starts in the spring, oblong chlorotic lesions from 8 to 15 mm. long appear on the lower leaves of infected plants. After about seven days lesions are light brown and dry at the center with a light red border. Numerous pycnidia appear in these dried areas after 12 to 14 days. The infected leaves turn yellow and often die prematurely. Severe leaf infection frequently results in almost complete defoliation. Ordinarily infection occurs only on localized spots on the leaves, but under favorable environmental conditions the infected areas may coalesce and cover large portions of the leaf surface. At such times infection often spreads to the sheath, stem, rachis, panicles, and glumes. Severe attacks stunt the plants and may even kill them. Selenophoma bromigena usually occurs alone on the host although other foliage diseases sometimes are found with leaf spot. Typical symptoms of leaf spot on B. inermis caused by S. bromigena are shown in figure 1, A.

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¹ Summary of a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy, granted by the University of Minnesota, June 1940.

Paper No. 2189 of the Scientific Journal Series, Minnesota Agricultural Experiment

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Description of the Pathogen

The pycnidia of Selenophoma bromigena are small, black, subepidermal, and globose with coarse globose peridial cells. They are 80 to 110 microns in diameter. The conidia are cylindrical, hyaline, slightly guttulate, non-septate, and multinucleate. They are 3 to 3.5 by 22 to 27 microns.

Host Range of the Pathogen

Selenophoma bromigena has been reported by Fischer (7) on Bromus This was the only report found in the literature listing S. bromigeng on any host other than B. inermis. To gain some knowledge of the host range the following species of brome and other grasses as well as a few cereals were tested for their reaction to artificial inoculation with the organism: Bromus anomalus Rupr., B. arvensis L., B. brizaeformis Fisch. and Mey., B. carinatus Hook. and Arn., B. ciliatus L., B. erectus Huds.. B. inermis Leyss., B. japonicus Thurb., B. laevipes Shear, B. lanuginsosus Pair, B. macrostachys L., B. madritensis L., B. marginatus Nees., B. mollis L., B. polyanthus Scribn., B. racemosus L., B. rigidus Roth, B. rubens L., B. secalinus L., B. tectorum L., B. vulgaris (Hook) Shear, Agropyron cristatum (L.) Gaerth, A. inerme (Scribn. and Smith) Rybd., A. trachycaulum (Link) Malte, A. repens (L.) Beauv., A. Smithii (Pursh) Scribn. and Smith, Elymus canadensis L., E. glaucus Buckl., Hordeum jubatum L., H. nodosum L., Poa compressa L., P. pratensis L., Sitanion hystrix (Nutt.) J. G. Smith, Hordeum vulgare L., barley varieties Glabron and Velvet, Avena sativa L., oat varieties Bond and Victoria, Secale cereale L., rye variety Rosen, and Triticum vulgare Vill., wheat varieties Marquis and Thatcher.

Inoculations were made in the greenhouse and in the field on young seedling plants and again on the same plants at about heading time by atomizing the foliage with conidial suspensions of the organism. Potted plants were inoculated, placed in a moist chamber for four days and then removed to a greenhouse held at approximately 65° F. Field plots were covered with muslin cages that were kept wet for four days following inoculation, and the muslin was then removed. Inoculations were made during cool, cloudy weather when the temperature did not exceed 70° F.

In these experiments *Bromus inermis* was the only host that became infected and developed lesions producing mature pycnidia.

Survival of the Pathogen

Septoria in leaf spots on cereals and forage grasses has been reported by Weber (11) to survive the winters by means of conidia retained in mature pycnidia. To determine how long the conidia of Selenophoma bromigena remain viable, dried foliage bearing many mature pycnidia was placed out-of-doors in a dry place. Germination of conidia was tested monthly. The rate of germination varied only slightly from month to month and a high percentage of conidia were still viable when the test terminated after 18 months. When mature pycnidia were placed in water drops on microscope

slides, conidia exuded in a gelatinous matrix, which fixed the conidia to the slides when dry. Conidia remained viable for six months on slides stored at 5° C. but less than one month at room temperature. Under natural conditions it is unlikely that conidia would be retained within their gelatinous matrix for long periods and the chances of their surviving variable weather conditions would be slight unless they remained inclosed in a pycnidium.

Dissemination of Inoculum

Many pycnidia dropped from mature lesions when the infected parts of the plant became dry and brittle. To determine if these pycnidia, free from plant material, were disseminated by the wind, microscope slides were coated with a thin film of vaseline and exposed on wind vanes at various distances from centers of heavy infection. Many pycnidia were collected on slides exposed one-half mile from any known infected plants, and it is probable that the pycnidia may be carried greater distances, accounting for much spread of the disease. Pycnidia and conidia are also splashed about by rain which accounts for local spread of the disease. The pycnidia may be seed borne in cases following severe infection when the fungus fructifies on the glumes, as Selenophoma bromigena has been isolated from such seed. The caryopsis of Bromus inermis is enclosed in the lemma and palea in commercially threshed seed and although floral bract infection is the exception rather than the rule it seems probable that the fungus is carried from one region to another in seed lots.

Germination of Conidia

Tests were made to determine the relationship of temperature to conidial germination and germ-tube elongation. Pycnidia scraped from natural lesions and from agar cultures were washed into tubes of sterile distilled water. Conidia were exuded from the pycnidia in large numbers. Drops of the conidial suspensions thus secured were transferred with a wire loop to cover slips inverted over van Tieghem cells in Petri dishes. A selected standard loop was used to transfer all drops to insure uniform size. The hanging drops of conidial suspensions were then incubated at temperatures ranging from 5° to 35° C. At regular intervals, 100 conidia were selected at random near the periphery of each drop and the germination was observed. The minimum temperature for conidial germination in distilled water was between 3° and 5°, the optimum between 19° and 22°, and the maximum between 30° and 35° C.

Above 30° and below 5° C., the germ tubes formed by conidia in water were very short and knotted. They often ruptured and extruded their protoplasm into the water drop. A similar phenomenon was reported by Caldwell (5) in germination studies of *Rhynchosporium*.

Conidia placed on the surface of drops of water agar inverted over van Tieghem cells and incubated at the optimum temperature, germinated after 12 hours. The conidia usually produced germ tubes from each end, or occasionally from one end, and the original spore remained recognizable for some time. Darley (6) reported that conidia of *Selenophoma bromigena* germinated on nutrient media form septate hyphal segments very early in the process of germination.

To determine if light was necessary for germination, suspensions of conidia in hanging drops were placed in complete darkness. Conidia germinated as readily in complete darkness as in daylight. Conidia were placed in varying dilutions of extracts made from leaves of susceptible and resistant brome plants but none of the extracts tried affected either the time required for germination or the final percentage of conidia that germinated.

PATHOLOGICAL HISTOLOGY

The relationship of Selenophoma bromigena to the host tissues and the development of the disease was studied microscopically, using leaves of Bromus inermis naturally infected in the field and infected as a result of artificial inoculation in the greenhouse. Stages in the development of the disease were followed from penetration of the host to the complete breakdown of the leaf tissue and fructification of the pathogen. Germination of conidia, formation of appressoria, and penetration of the cuticle were studied in segments of whole leaves cleared in acetic alcohol and stained with methyl blue. The conidia and germ tubes were heavily stained while the host tissue remained clear.

Penetration was observed through both upper and lower surfaces of the leaf, but more infection occurred on the upper surface. Conidial germ tubes were never observed to penetrate the leaf through the stomata. In fact, germ tubes were observed growing across stomata to form appressoria elsewhere on the epidermal wall. The germ tubes formed small rounded structures at their ends and these functioned as appressoria. The cuticle was penetrated within 48 hours. The penetrating hyphae ramified through the subcuticular region and between the cells of the epidermis. The outer epidermal wall collapsed first, then followed breakdown of the entire epidermal cell. The mycelium then began to grow intercellularly into the mesophyll from the stroma that had been formed. Mesophyll cells broke down rapidly, often slightly in advance of the invading mycelium.

The earliest evidence of infection appeared 7 to 8 days after inoculation, when chlorotic areas were noted at the points of infection. The time required for reproduction of the pathogen, from inoculation to formation of pycnidia containing mature conidia, was from 12 to 15 days.

Fructification occurred after the complete breakdown of the leaf tissue in the infected spot. During the invasion and breakdown of the mesophyll the subcuticular mycelium developed rapidly and formed a stroma several cells thick beneath the ruptured cuticle. The pycnidia were formed superficially among the cells of the stroma after the mycelium had advanced into the mesophyll. Pycnidia were abundant in the central and completely collapsed area of the leaf spot. The fungus did not grow through the leaf

to fruit on the opposite surface. Fructification on both surfaces of a given leaf area occurred apparently only when separate infections developed on opposite sides of the leaf.

FACTORS AFFECTING DISEASE DEVELOPMENT

The Selenophoma leaf spot appeared early each spring. Heavily infected leaves of *Bromus inermis* with lesions bearing newly formed mature pycnidia were collected from nursery rows on March 15, 1938, at St. Paul. The disease developed rapidly during April, May, and early June. During the summer there were relatively few leaf spots, but many appeared during September and October.

In controlled tests the optimum temperature and humidity relationship for infection and development of the disease were determined. The optimum temperature ranged between 15° and 25° C. A film of water on leaves at the time of infection and continued high humidity favored rapid development. Above 28° or below 8° C. only slight infection resulted from artificial inoculation, and the development of the disease was noticeably checked when the temperature rose above 30° C. Thus cool, moist conditions were necessary for infection, rapid development, and spread of the disease.

PHYSIOLOGIC SPECIALIZATION

Monoconidial cultures of *Selenophoma bromigena* isolated from diseased smooth brome plants collected from various regions in Minnesota were tested for pathogenic differences. Seedling and adult plants from seed of openpollinated and selfed lines of *Bromus inermis* were artificially inoculated. One culture caused severe infection on seedling and adult plants from openpollinated lines whereas the cultures from the other regions, although pathogenic, caused much less infection. The culture that was most pathogenic on open-pollinated lines was also pathogenic on selfed lines which were resistant to the other cultures.

These results indicate that at least two pathogenically specialized races of *Selenophoma bromigena* exist in nature. Differentially parasitic races in the genus *Septoria* have been reported by Beach (3), but this is the first known record of physiologic races in the genus *Selenophoma*.

Culture Study

Several hundred monosporous isolates of *Selenophoma bromigena* were cultured during this investigation. Single conidia were isolated by means of a micro-manipulator. Each conidium was placed on a small sterile drop of potato-dextrose agar on the lower surface of a cover slip mounted on a van Tieghem cell. When germinated, usually within 48 hours, each isolate was transferred to a 125-cc. Erlenmeyer flask containing 30 cc. of potato-dextrose agar (390 g. potatoes, 20 g. dextrose and 15 g. agar per liter).

When cultures were grown at temperatures ranging from 5° to 30° C. the optimum for growth of the fungus was between 20° and 25° C. As this

range approximates room temperature all later culture studies were at room temperature.

All cultures produced aerial mycelium sparingly and growth consisted of intertwining mycelium which formed tough, leathery mycelial mats that often became rough and convoluted. Pycnidia first appeared in the central part of the mat after 15 to 20 days and gradually formed over the entire

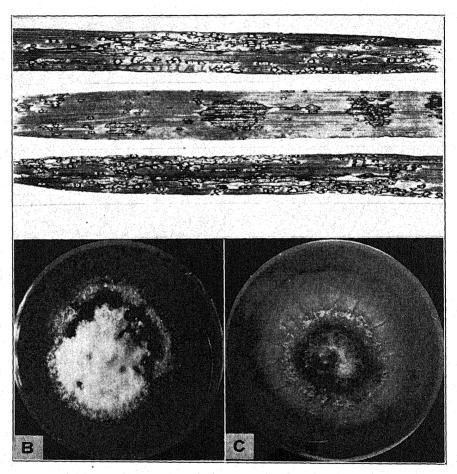


Fig. 1. Selenophoma bromigena. A. Typical lesions showing pycnidia on blades of smooth bromegrass. B. A monosporous culture showing the development of a mycelial variant. C. A morphologically distinct variant produced by a monosporous culture.

mycelial surface. Conidia were abundant. All cultures produced water-soluble pigments that diffused into the medium in advance of marginal mycelial growth. The perfect stage was not produced in culture.

Monosporous cultures were much the same in their general morphology, although there appeared to be some geographic relationship in cultural characteristics among isolates taken from specimens collected at specific localities. This phenomenon has been described by the writer (2).

Occasional cultures varied considerably in their general morphology and could easily be distinguished from the ordinary type of culture. Fanshaped patches or sectors, which lacked pycnidia, characterized these cultures (Fig. 1B). Mycelial subtransfers, made by cutting small bits of mycelium from the margins of sectors, were placed on flasks of potato-dextrose agar. Each subtransfer developed the characteristics of the variant from which it was taken. Variant subtransfers produced no pycnidia or conidia and could only be perpetuated by mycelial transfer. Morphologically distinct variants were often obtained from different monosporous cultures (Fig. 1C). Variants remained stable through repeated subtransfer on potato-dextrose agar for over two years.

Variant cultures were nonpathogenic. As no spore form was produced by the variant cultures, mycelial mats were macerated and hypodermically injected into the leaf curls of known susceptible smooth brome plants but no infection was obtained. When plants were inoculated with a conidial suspension of *Selenophoma bromigena* by this method excellent infection resulted.

SUMMARY

Selenophoma bromigena causes a leaf spot on Bromus inermis. The disease is general in its distribution, appearing wherever smooth brome is grown. The fungus appears to be specific in attack, producing disease symptoms only on B. inermis of the many brome species. Other grasses and cultivated cereals were not infected in artificial inoculation trials. The disease is most prevalent during the spring and develops best in moist, cool weather. No perfect stage of the fungus has been found. The fungus apparently overwinters in the pycnidial stage. Penetration of the host tissues is direct. The fungus is disseminated by the wind and rain and by infected seed. Smooth brome plants resistant to the fungus are common, and selection and breeding present a desirable means of control. Specialized races of the fungus exist in nature, as two distinct parasitic races were isolated.

Monosporous isolates of *Selenophoma bromigena* grew best on potato-dextrose agar between 20° and 25° C. and were uniform in their cultural characteristics. Occasional cultures produced sectors or variants. Variants did not produce pycnidia or conidia and could only be perpetuated by mycelial subtransfers. Variants were stable in culture, remaining constant for two years through repeated subtransfer. Variant type cultures were nonpathogenic.

AGRICULTURAL EXPERIMENT STATION, MADISON, WISCONSIN.

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THE BROWN LEAF SPOT ON BROMUS INERMIS CAUSED BY PYRENOPHORA BROMI¹

D. W. CHAMBERLAIN² AND J. LEWIS ALLISON³

(Accepted for publication November 17, 1944)

INTRODUCTION

A brown leaf spot was observed in the spring of 1941 on smooth bromegrass (Bromus inermis Leyss.) in the grass nurseries at the Wisconsin Agricultural Experiment Station at Madison. This paper presents a study of the life history of the causal organism, its relationship to the host and the factors influencing the development of the disease.

THE DISEASE

History

The causal organism was identified as Pyrenophora bromi (Died.) Drechsler (Helminthosporium bromi Died.). The first description of the disease is in Diedicke's (1) report from Germany in 1902. He described the fungus as a parasite on Bromus asper Murr., believing it to be a biological species of Helminthosporium gramineum Rab. Later (2) he recognized it as a distinct species, H. bromi, and also described the ascigerous stage as Pleospora bromi on the same host. Krieger collected H. bromi on Bromus inermis Leyss.

Drechsler (3) contributed morphological details by which Helminthosporium bromi could be distinguished from H. teres Sacc., a distinction not previously made by Diedicke whose conidial measurements rendered the two species indistinguishable. Drechsler also transferred the ascigerous stage to the genus Pyrenophora Fr., which has bristles on the perithecium, distinguishing it from *Pleospora* Rab. which has a smooth perithecium. While the former genus has not been accepted universally, the distinction has been recognized as valid by Saccardo (6) and Lindau (4). Accordingly, the causal organism will be termed Pyrenophora bromi (Died.) Drechsler throughout this paper.

Symptoms

The symptoms of the disease are essentially those described by Diedicke and corroborated by Drechsler. Minute, dark brown, scattered specks appear on the young leaf blades. A yellow halo gradually develops around each spot. Both the central spot and the surrounding halo increase in size,

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² Formerly Research Assistant in Plant Pathology, University of Wisconsin.

³ Associate Pathologist, Division of Forage Crops and Diseases, and Assistant Pro-

fessor of Plant Pathology, University of Wisconsin.

4 Krieger, W. Fungi Saxonici. No. 1941 Helminthosporium bromi Died. (Exsiccati). 1903, 1905.

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especially longitudinally, and coalescing lesions frequently form large, yellowed patches on the leaf (Fig. 1, left). These symptoms are followed by a general yellowing of the leaf from the tip downward until the entire leaf withers. Conidiophores may be produced on or near the individual brown spots or generally over the surface of the withered leaf.

Although Pyrenophora bromi usually occurs alone on Bromus inermis, it is sometimes found with other pathogens.

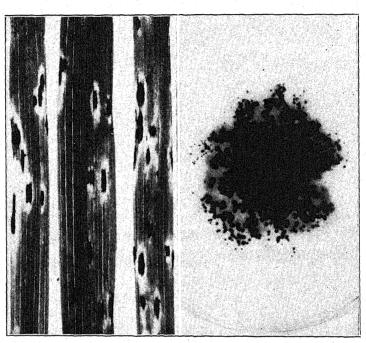


Fig. 1. Pyrenophora bromi. Left. Typical symptoms on Bromus inermis. Right. Perithecial initials produced on potato-dextrose agar, as seen from the under side of the Petri dish. Photographs by Eugene Herrling.

Description

The conidia produced by $Pyrenophora\ bromi$ were described by Diedicke, whose description was later revised by Drechsler. In his original report from Germany, Diedicke stated that the conidia of the fungus measured $108-150\ \mu\times13-20\ \mu,$ with 4–6 septa. Working with material collected at Madison, Wisconsin, Drechsler was unable to substantiate these measurements. He found that conidia measured $45-265\ \mu\times14-26\ \mu,$ with 1–10 septa, and that the contour of the basal cell was hemiellipsoidal.

Conidial measurements made by the writers agreed substantially with those of Drechsler. Typical conidia from host plants in the field and from potato-dextrose-agar cultures varied from 57 to 201 μ in length, and from 13 to 19 μ in diameter. Under certain conditions, however, the fungus produced much larger conidia. When diseased leaves were removed from plants and maintained for 2 or 3 days in a Petri-dish moist chamber, conidia of atypical length, measuring as much as 400 μ , were produced.

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According to Drechsler the mature perithecium is a dark brown subglobose body 0.3–0.4 mm. in diameter and embedded in the leaf tissue, and it has an irregular ostiolar beak which protrudes above the broken epidermis of the leaf. A variable number of septate, sterile bristles were observed near the tip of the ostiolar beak. The ascus was described as having a short stipe at the base and a characteristic ring-like thickening at the apex, with the light brown ascospores borne in distichous arrangement in the granular epiplasm. The ascospores, uniformly divided by 3 transverse septa, measured $20{\text -}30~\mu \times 45{\text -}72~\mu$.

Little could be added to this description except in the way of confirmation. Asci were from 190 to 300 μ long, and from 36 to 60 μ wide. Ascosporic measurements were 48–77 $\mu\times22$ –30 μ . The segments of the ascospore were commonly divided by one or two longitudinal septations, and a gelatinous matrix surrounded each spore after discharge from the ascus.

HOST RANGE OF THE PATHOGEN

Pyrenophora bromi has been reported as attacking only Bromus inermis in North America. Host range studies made during this investigation included 6 grasses and 4 cereals common to Wisconsin: quackgrass (Agropyron repens (L.) Beauv.), redtop (Agrostis alba L.), smooth bromegrass (Bromus inermis Leyss.), timothy (Phleum pratense L.), Canada bluegrass (Poa compressa L.), Kentucky bluegrass (Poa pratensis L.), barley (Hordeum vulgare L.), oats (Avena sativa L.), rye (Secale cereale L.), and wheat (Triticum vulgare Vill.). Tests were made in the greenhouse with seedling plants, each species receiving 4 inoculations with ascospores of Pyrenophora bromi. The plants were held in a moist chamber for 4 days, until B. inermis developed typical brown-spot lesions, and then were incubated for another 7 days on the greenhouse bench.

Only *Bromus inermis* developed typical brown-spot symptoms and produced lesions from which the fungus could be reisolated. Inbred lines of *Bromus inermis* differed in reaction to brown spot. Some lines were completely susceptible to the disease while others were highly resistant.

OVERWINTERING OF THE PATHOGEN

Perithecia appear to be the only means of overwintering the fungus in Wisconsin. These structures are abundant in the leaf tissue, becoming macroscopically visible as early as June 4 at Madison. Although perithecial development was studied continuously through the summer and fall, no asci were ever found delimited during the current season. The low temperatures of late fall and winter are necessary for maturation of the perithecia. During the subsequent spring mature asci can be found in the perithecia on overwintered leaf material lying on the ground.

In order to check these observations, dried leaves containing immature perithecia were collected and divided into 3 lots. One lot was kept dry at 20° C., the second was dry at 10° C., while the third was on moist filter

paper at 10° C. After 3 months, perithecia from all 3 lots were crushed and examined for ascospores. The importance of low temperature and moisture was emphasized by the results: ascospores were found only in the third lot, maintained at 10° C. and supplied with moisture.

Experiments were made to determine other possible means of overwintering the fungus. Heavily infected plants on which no perithecial initials had developed were removed from the nursery and potted early in November. These plants were allowed to freeze and were held out-of-doors throughout the winter. In early April, dried leaves were collected from them, surface-sterilized in a 1 to 10 solution of sodium hypochlorite for varying intervals, and plated on potato-dextrose agar to determine the possibility of mycelium overwintering in the leaf tissues. *Pyrenophora bromi* was never recovered in any of these platings. When the overwintered plants resumed growth in the spring, there was no evidence of disease on the new leaf blades.

Several lots of conidia on glass slides were held out-of-doors at 15-20° F. for 3 days. The slides were then moistened and incubated at 20° C., but none of the conidia germinated. Evidently the thin-walled conidia cannot survive the winter in Wisconsin.

LONGEVITY OF SPORES

In considering the importance of the ascigerous and the imperfect stages of the fungus, it seemed advisable to determine the comparative longevity of ascospores and conidia. Field collections of sporulating material were brought into the laboratory, and spore germination was tested each subsequent day. Conidia on dried leaves remained viable for a maximum of 11 days after storage in the laboratory. Conidia that were mounted in water on glass slides, allowed to dry, and stored in the laboratory remained viable for a maximum of 9 days. This experiment was repeated at various times with 6 different lots of spores.

Ascospores had greater longevity than conidia. Perithecia from overwintered leaves, after storage in the laboratory for 7 months, yielded viable ascospores. Crushed perithecia were mounted on glass slides and stored in a dry state to test the longevity of discharged ascospores. Although drying for more than 2 weeks resulted in reduced percentages of germination, 50 per cent of the spores germinated after 26 days, the longest period for which tests were made.

SPORE GERMINATION

Ascospores and conidia germinated over a wide temperature range, with the high and low levels being the same for each spore form. The optimum for each, however, differed by 8° C.

Ascospores and conidia in hanging drops of distilled water were placed in controlled temperature cabinets, set at 4-degree intervals, from 0° to 36° C. All mounts were observed every 2 hours for 20 hours. Neither spore form

germinated at 0° or 36° C. within 20 hours. The optimum temperature for ascospore germination, based on the number and length of germ tubes, was 20° C. and germination began within 2 hours. The optimum for conidia was 28° C. with germination beginning within 2 hours. Germination of both spore forms was equally good in light or darkness.

DISSEMINATION OF INOCULUM

Ascospores are discharged readily from the perithecium in the presence of moisture and may be further distributed by wind, as indicated by spore-trapping experiments. Glass slides, with approximately 9 sq. cm. of surface covered with a thin film of vaseline, were mounted in centers of infection at the level of developing brome blades (approximately 3–6 inches) during the last week in April and the first week in May, 1942. Slides were replaced and examined each day. Slides exposed for 24 hours following rainfall had from 7 to 10 ascospores per slide, while slides exposed 40 hours after rainfall had no spores.

Conidia apparently play a minor rôle in the spread of the disease during periods favorable for spore production. Conidial production at Madison is extremely sparse over the growing season of the host, occurring only during cool, wet periods. In spite of constant search for sporulating material during two years, the only conidia found in any abundance were collected during October, 1942. In the spore-trapping experiments, few conidia were caught; the maximum number ever obtained on a single slide was 3.

Since some members of the genus *Helminthosporium* are known to be seed-borne, this possibility was tested in the case of *Pyrenophora bromi*. Seeds were collected from the panicles of heavily infected plants and at one-month intervals for six months were plated on potato-dextrose agar. Although this experiment was repeated with 6 different lots of seeds, *P. bromi* was not recovered in any of the platings.

PATHOLOGICAL HISTOLOGY

In studies of fungus-host relationships, portions of the leaf were cleared for 48 hours in a mixture consisting of equal proportions of acetic acid and absolute alcohol. Cotton blue and lacto-phenol were satisfactory for staining and mounting these preparations and were especially good for demonstrating early stages of infection. In studying older lesions, well-pressed leaf specimens could be sectioned dry with the aid of a sharp razor blade and mounted directly in cotton blue diluted with lacto-phenol. The fungus in the host tissues stains a deep blue, while little if any of the stain is taken up by the leaf cells.

To determine the method of penetration, plants were sprayed with ascosporic inoculum and incubated in a moist chamber. Each day thereafter, leaves were removed from these plants, cleared, stained, and examined microscopically.

The organism enters the host by direct penetration of the epidermal cells.

SH

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b

The germ tube from the ascospore grows over the leaf surface and produces an appressorium at its tip. The infection peg penetrates the epidermal cell directly through the uninjured cuticle. No evidence of stomatal penetration was ever encountered, the germ tubes frequently passing over and beyond stomata to form appressoria elsewhere on the leaf surface.

After penetration, the fungus establishes itself in the intercellular spaces of the epidermal and mesophyll tissues and ramifies freely between the mesophyll cells. The epidermal cells collapse soon after invasion and within 3 days the mesophyll cells collapse and turn brown. At this time the lesion is macroscopically visible as a small brown spot surrounded by a water-soaked area. The progress of the fungus is somewhat limited by the vascular bundles, accounting for the elongate contour which is typical of most well-developed lesions. Browning and collapse of the cells progress from the upper to the lower leaf-epidermis so that the lesion is visible from beneath the leaf 4 days after inoculation. Conidiophores emerge singly or in pairs between the upper epidermal cells, usually after the leaf has withered.

Perithecia are formed in the mesophyll, the leaf tissues becoming distended as the initials are formed. Further growth of the perithecium ruptures the leaf epidermis, exposing the beak.

FACTORS AFFECTING DISEASE DEVELOPMENT

Like most of the leaf-spot diseases of smooth bromegrass, brown spot is favored by cool weather and abundant moisture. Its most destructive phase appears during the spring and reaches a peak about the first of June in Wisconsin. Through the hot, dry portion of the summer there is relatively little, if any, spread of the disease. Rain is necessary for ascosporic discharge and spore germination. Cloudy periods lasting for several days, with intermittent rainfall, are especially conducive to new outbreaks of infection.

These observations were corroborated by controlled experiments performed in the greenhouse. Inoculated plants growing in pots were held under moist-chambers for 3 days at 4°, 16°, 20°, 24°, and 28° C. Duplicate lots of plants for each treatment were used in 4 different trials. Three days after inoculation the moist chambers were removed and the lesions allowed to develop for another three days. On the basis of the number of lesions formed and the size of individual lesions, the disease always developed best between 16° and 20° C. No lesions developed at 4° C. Moisture, either in the form of a continuous film or a high air humidity, was necessary because in parallel experiments without moist-chamber treatment or a film of water after inoculation no infection occurred.

LIFE HISTORY OF THE ORGANISM IN RELATION TO DISEASE DEVELOPMENT

From the standpoint of disease development, the ascigerous stage is the most important one in the life history of *Pyrenophora bromi*. Ascospores can be found very early in the spring in overwintered perithecia, formed during the previous year. Such perithecia provide the abundant initial

inoculum and also remain as potential sources of inoculum throughout the spring.

Conidia are borne on the initial lesions or, more frequently, on the discolored tips of infected leaves during periods of cool weather and high humidity. Although they cause some infections, conidia are produced so infrequently and in such sparse quantities that they are of less importance in the disease cycle than ascospores. The conidia are short-lived and do not survive the winter in Wisconsin.

Perithecia are formed in the leaves during the summer but do not produce mature ascospores until the following spring. Thus *Pyrenophora bromi* requires one year to complete its life cycle.

CULTURAL STUDIES

Growth of the fungus was slow on all artificial media. A number of media were tried, but none was superior to potato-dextrose agar composed of 200 grams of peeled potatoes, 25 grams of agar, and 20 grams of dextrose per liter of distilled water.

According to Drechsler, cultures derived from conidia differed in no way from those derived from ascospores, and the appearance of black sclerotial bodies within 12 days characterized the growth of the fungus on agar. Repeated monosporous cultures of both conidia and ascospores made during this investigation verified these statements. The black sclerotial bodies, actually perithecial initials, become visible on the underside of the culture within 8–9 days after isolation (Fig. 1, right).

On potato-dextrose agar the fungus is at first white and fuzzy, later becoming gray to buff. The aerial growth is short. A culture 20–30 days old usually appears as a rough disc, with a raised periphery and a somewhat sunken central portion, covering approximately three-quarters of a Petri dish. The fungus appears to be stable on potato-dextrose agar, since no sectoring was observed.

In an effort to induce the production of conidia, always extremely sparse on agar, cultures of *Pyrenophora bromi* growing on potato-dextrose agar were exposed to continuous fluorescent light for 5–7 days. Some increase in sporulation was noted, and cultivation of the fungus on vegetable agar (5) accomplished the same result, although neither of these treatments resulted in what might be termed abundant conidial production as judged by any fungus that sporulates well in culture.

Since there is no record of the production of the ascigerous stage of *Pyrenophora bromi* on artificial media, various treatments were applied to cultures in an attempt to bring the perithecia to maturity. Subjecting cultures 3 to 4 weeks old to low temperatures for 3 months resulted in the production of mature ascospores which readily produced the characteristic disease symptoms when transferred to susceptible plants. As a control, several cultures were kept at laboratory temperature but no ascospores were produced.

SII

Perithecia formed in culture do not discharge their contents readily. The asci, being tough and elastic, are very difficult to rupture. germinate readily within the ascus, however, and produce infection on susceptible plants. Asci and ascospores produced in culture differ in no respect from those formed in nature on leaf-tissue, except that the spores formed in culture are at times almost hyaline. The perithecia on potato-dextrose agar are not always similar in shape to those on the host leaf, but the production of bristles on the perithecium is consistent. Not all asci within a single perithecium mature at the same time, and all stages of development, from asci with spores undelimited to those containing 8 mature spores, may be found.

In order to determine the range of temperatures at which asci mature, cultures that had been grown for 3 weeks at laboratory temperature were incubated at 4°, 8°, 10°, 12°, and 20° C. for 3 months. No ascospores were produced at temperatures above 12° C.

Pyrenophora bromi is homothallic, since perithecia bearing mature asci and ascospores were produced in single-ascospore cultures.

SUMMARY

The causal organism of the brown leaf spot on Bromus inermis was identified as Pyrenophora bromi. Host range studies demonstrated that the fungus is specific to B. inermis. There are indications of differences in disease reaction among inbred lines of B. inermis in Wisconsin.

Pyrenophora bromi overwinters in the perithecial stage. Ascospores are the important inoculum; conidia are fragile, sparsely produced, and shortlived. Both spore forms germinated at temperatures ranging from 4° to 32° C., the optimum for ascospore germination being 20° C. and that for conidia 28° C.

The fungus is homothallic. The ascigerous stage was produced repeatedly by subjecting mono-ascosporic cultures to low temperatures for 3 months.

Pyrenophora bromi invades the host by direct penetration of the leaf epidermis and ramifies intercellularly in the leaf tissues. The development of the disease is favored by cool weather and abundant moisture.

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ALTERNARIA RICINI (YOSHII) HANSFORD, THE CAUSE OF A SERIOUS DISEASE OF THE CASTOR-BEAN PLANT (RICINUS COMMUNIS L.) IN THE UNITED STATES

E. C. STEVENSON1

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INTRODUCTION

Castor oil is used in the production of paints, varnishes, textiles, leather. hydraulic fluids, soap, medicine, linoleum, printing ink, lubricants, and many miscellaneous products.

With the entrance of the United States into World War II, imports of castor beans and oil were seriously curtailed, while at the same time demand for these materials increased. Domestic production of castor beans was immediately contemplated but no quantity of reliable seed-stocks was available, consequently variety tests and seed-increase programs were begun by the United States Department of Agriculture.

In the course of a variety and adaptation test at the Plant Industry Station, Beltsville, Maryland, in 1942, a fungus of the genus Alternaria was found fruiting extensively on ripe capsules of the castor-bean plant. the racemes were mature when this fungus was first noted, the organism was thought to be a saprophyte or an extremely weak parasite. However, during the seasons of 1943 and 1944 the same fungus caused serious damage to the seedlings, inflorescences, and leaves of the castor-bean plant.

Other workers have previously encountered species of Alternaria and Macrosporium on the castor-bean plant. Cooke (5) described M. compactum on mature stems of plants in Texas. Dastur (6) and Chibber (4) observed an Alternaria leaf spot in India. Parisi (12) described M. cavarae causing a leaf spot and seedling disease in Italy. Tropova (13) reported M. cavarae on the leaves, M. nigricans Atk.2 on the racemes and A. tenuis Nees on the stems of plants in Russia. Tropova (14) again reported M. cavarae on the leaves in Russia; Kvashnina (9) reported M. cavarae on the cotyledons, leaves, and racemes in Russia. Yoshii (16) described a leaf spot in Korea and Japan and demonstrated by inoculation experiments that the causal organism was a species of Macrosporium which he named M. ricini. Baldacci (2) isolated M. cavarae from seed apexes in Italy and attributed leaf-spotting to this organism. Golovin (7), reported M. cavarae on the leaves, racemes, and capsules and A. tenuis on the stems of castor-bean plants in Russia. Bitancourt (3) found species of Alternaria causing leaf spots in Brazil. Weiss (15) reported species of Alternaria

² The binomial as given by Atkinson is M. nigricantium.

¹ Associate Plant Pathologist, Division of Drug and Related Plants, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland. Acknowledgment is made of assistance given by J. A. Stevenson, Edith K. Cash, and Jessie Wood of the Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration.

² The binomial as given by Atkinson is M. nigricantium.

associated with leaf spots in Florida, Louisiana, and Texas, and A. brassicae (Berk.) Sacc. causing a leaf spot in New York. McClellan (10) reported A. compacta (Cke.) McClellan attacking castor-bean seedlings in the greenhouse. Hansford (8) changed the designation of Macrosporium ricini to Alternaria ricini.

SYMPTOMATOLOGY

Seedlings. The condition of Alternaria-diseased seedlings can be best described as a die-back or blight. The cotyledons are stunted, spotted, and malformed. If the infection is extensive, the seedling dies but if only the

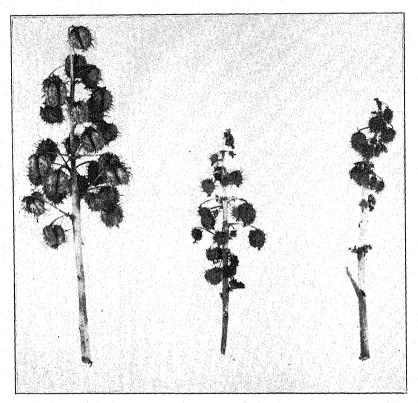


FIG. 1. Alternaria ricini on the racemes of Ricinus communis (variety U. S. No. 4). Left to right, normal raceme, partially affected raceme, and heavily affected raceme.

tips of the cotyledons are affected the seedling survives but its growth is retarded. Often the spores of the causal organism can be found on the surface of injured cotyledons.

Inflorescences. The first infections of the inflorescences seem to be confined to capsules which have attained half or more of their ultimate size. Two types of symptoms can be found. In the first the capsules wilt suddenly, turn purple or dark brown (depending on the host variety), the pedicels collapse, the seed is usually poorly filled, and normal dehiscence fails to take place (Fig. 1). In the second type of infection a sunken area

develops on one side of the capsule and this area enlarges until the whole capsule becomes involved. In this case poorly-filled seed is not prevalent and no great interference with dehiscence occurs. As the disease spreads and large quantities of inoculum are produced, inflorescences in any stage of development are attacked. In heavily infected areas all of the young racemes and even flower primordia are killed. Following periods of high humidity the fungus fruits extensively on the surface of infected inflorescences, giving them a black, sooty appearance (Fig. 2). When the first harvest was made at Beltsville, Maryland, on September 6, 1943, the spores

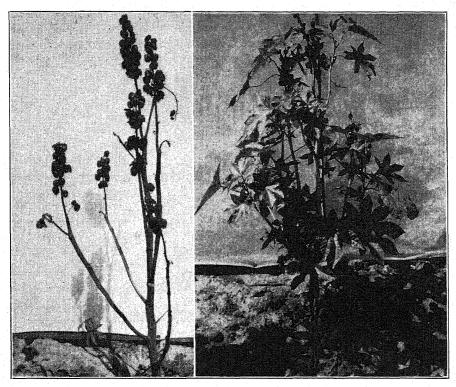


Fig. 2. Left, defoliation of a selection of Ricinus communis following severe leaf infection by Alternaria ricini. Right, an adjacent selection of R. communis with leaves only slightly affected. Note the blackening of capsules of both plants caused by the presence of mycelium and spores of A. ricini.

of Alternaria rose in clouds of dust from the capsules, and workers' hands became black with spores. The number of plants visibly affected by September 15 was determined on an area of approximately five-sixths of an acre. Of a total of 3,603 plants, 70 per cent were affected to some degree.

Leaves. A certain amount of leaf spotting and premature defoliation was noted throughout the season. As the raceme infection spread this leaf spotting and defoliation became increasingly severe. By August 31, 1943, some selections were almost completely defoliated while others were little affected (Fig. 2). Diseased leaves dry and curl at the edges and scattered

brown spots, which often coalesce, appear over the surface. When sufficient humidity is present the fungus fruits extensively over the infected portion of the leaf. When a large area of the leaf becomes involved, abscission follows.

THE CAUSAL ORGANISM

Morphology. Material for determining the spore characters and dimensions of the causal organism was collected immediately following periods of high humidity from naturally infected field-grown capsules on which the sporulation was fresh. The conidia and conidiophores were mounted in lactophenol, measurements being made with an ocular micrometer. Only mature spores were measured.

The description of the organism is as follows: Conidiophores solitary or in fascicles, simple or branched, olivaceous in plain water and lactophenol,

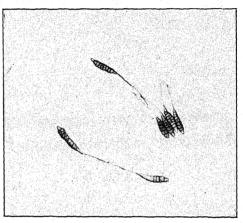


Fig. 3. Conidia of Alternaria ricini (Yoshii) Hansford, scraped from the surface of an infected easter-bean capsule. (×80.)

becoming yellow-green after a short time in lactophenol, length 70–128 $\mu,$ diameter 4–6 $\mu,$ with 5–10 septa; conidia obclavate, dark olive-green en masse and light-olive when viewed singly in either water or lactophenol (if stored in lactophenol the conidia become cinnamon-brown en masse and straw-colored when viewed individually). The conidia exclusive of the beak are 47–96 μ long, with a mean of 70 $\mu,$ and 15–29 μ wide, with a mean of 22 $\mu.$ There are 5–12 transverse septa, with a mean of 8, and 0–2 longitudinal septa, with a mean of 1.6. The conidia have hyaline beaks which are 51–200 μ long, with a mean of 112 $\mu.$ Branching of the beak was observed in only one case. Typical conidia are shown in figure 3.

TAXONOMY

The differentiating characters of the fungus were compared with the other species of *Macrosporium* and *Alternaria* reported as occurring on the castor bean. This fungus differs from *M. compactum* Cke. (A. compacta (Cke.) McClellan) and *M. cavarae* Parisi in that the conidia have long beaks

and are considerably larger than those of the latter two species. M. cavarae, as described by Tropova (13, 14), Kvashnina (9), and Golovin (7), agrees well with the Alternaria found by the writer but fails to fit Parisi's original description of M. cavarae. M. nigricans (M. nigricantium Atk.) and A. tenuis Nees as described and illustrated by Tropova (13) are much like the Alternaria under discussion but these descriptions also fail to agree with the original descriptions of these species as given by Atkinson (1) and Nees (11) respectively. A. tenuis as reported by Golovin differs both from the Alternaria of the writer and the description of A. tenuis as given by Nees (11). M. ricini (A. ricini (Yoshii) Hansford) as described and illustrated by Yoshii (16) appears to be the same fungus as that reported herein. The conidia as described by Yoshii tend to be slightly shorter than in the species under study but this variation is thought not to be sufficient to justify giving this fungus a new specific designation. Considering the extreme variability within any one species of the genus Alternaria, it is conceivable that the same fungus might have been the cause of the disease in several and perhaps all of the instances reported in the literature, but the material is not available and it is not within the scope of this article to attempt to reduce these species to synonymy. However, because of the ambiguity in the use of the binomials suggested prior to Yoshii's work, they may be disregarded in the present study and the binomial A. ricini (Yoshii) Hansford accepted for the fungus herein described.

EXPERIMENTAL TESTS

In addition to the large numbers of spores of *Alternaria ricini* observed on infected capsules and leaves, repeated isolations from affected organs consistently yielded this fungus.

Reliable artificial infections of capsules were obtained only by wounding the surface of each capsule with a sterile needle and introducing the Alternaria spores and mycelium into the wound. The symptoms produced on inoculated capsules were identical with those observed as a result of natural infections. The causal organism was re-isolated readily from the seed coat, the central column of the capsule, and other capsular material. The results of typical inoculations are summarized in table 1. On controls treated in a like manner with the absence of the causal organism no symptoms developed and capsules ripened normally.

Castor-bean plants for leaf inoculations were grown in 5-inch pots in the greenhouse or in soil on a greenhouse bench. Plants were inoculated when in the sixth true leaf stage. Suspensions of spores and mycelium were made by dispersing the fungal mat of single-spore cultures in sterile water using a Waring Blendor. The plants were atomized with this suspension and placed in a moist chamber or covered with a large bell jar. In this humid atmosphere symptoms were visible in two days. The plants were removed from these moist conditions after 2 to 4 days but the fungus, once established, continued to attack the leaves, petioles, and leaf scars, causing the

leaves to absciss prematurely. Control plants, sprayed with water only, showed no symptoms. An inoculated leaf is shown in figure 4. The fungus was recovered readily from inoculated leaves and typical spores were produced on these leaves in the greenhouse, when the humidity was maintained at a high level. Results of leaf inoculations are shown in table 1.

Seed from heavily infected capsules nearly always carries the fungus either on the seed coat, caruncle, or endosperm. In order to determine the subsequent effect of this seed-borne fungus on the seedlings, seed from infected capsules of the varieties, Conner and Doughty 11, was planted in sterilized soil in the greenhouse. After emergence the plants were examined for lesions and malformations. Seventy-two per cent of the seedlings of the Conner variety and 17 per cent of the variety Doughty 11 had symptoms

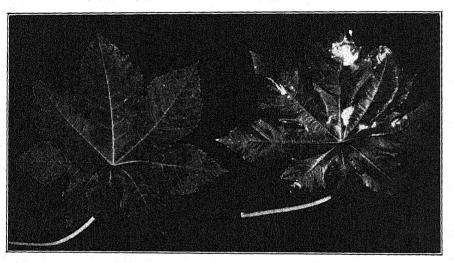


Fig. 4. Right, leaf of Ricinus communis (variety Kentucky 38) following inoculation with Alternaria ricini. Left, a normal leaf.

typical for Alternaria ricini. One hundred of these seedlings with stem lesions were taken at random and the diseased portions plated on acid potatodextrose agar. A. ricini was readily recovered from these diseased seedlings but the extreme lack of fruiting of this fungus under most cultural conditions made it impossible to check the spore size and type from agar plates. Therefore, 100 diseased seedlings were washed thoroughly in tap water and placed in moist chambers. After 3 to 5 days the lesions were examined for spores. Mycelium, but no spores, was found on 25 seedlings, Fusarium spores were found on 23 seedlings, and A. ricini spores of the type produced on naturally infected capsules and leaves in the field were found on 52 seedlings.

DISTRIBUTION OF THE DISEASE

Isolates of Alternaria, having the cultural characteristics of A. ricini, were obtained by the writer from 32 of 34 samples of castor-bean seed from

TABLE 1.—Infection of capsules and leaves of the castor-bean plant following inoculation with Alternaria ricini

Plant numbers and	No. capsules		No. infected	
part inoculated	or leaves — inoculated	Heavily	Moderately	Lightly
Capsules—field				
44–1	10	9	0	1
44–2	10	9	0	1
44–3	8	5	3	0
44–4		11	1	0
44–5		4	9	0
44-6		6	3	0
44–7		0	9	2
44-8		13	5	0
44–9		14	6	0
44–10		0	0	1.5
Check-1		0	0	0
Check-2		0	0	0
Check-3		0	745.00	0
Check-4		0	0	0
Check-5	15	0	0	0
Capsules—greenhouse				
45–1		15	0	0
46-1		8	0	0
46-2		4	0	0
46–3		2	0	0
46–4		6	0	0
Check-6		0	0	0
Check-7	18	0	0	0
Leaves—greenhouse				
47-1		3	1	1
47-2		3	0	2
47–3		3	1	1
47–4		3	1	1
47–5		5	0 1 2	0
47-6		4	1	1
47-7		2	0	2
Check-8		0	0	0
Check-9		0	0	0
Check-10		0	0	0
Check-11	5	0	0	0

^a All plants were of the variety, Kentucky 38, except plants 46-1, 2, 3, 4, Check-6, and Check-7, which were variety number 227, a spineless variant of variety U. S. 4.

the 1942 crop, representing 34 different locations in the United States. That *A. ricini* was widespread on the castor-bean plant in the United States in 1943 is evidenced by the fact that spores of *A. ricini* were abundant on the surface of capsules from Texas, Louisiana, Mississippi, Georgia, South Carolina, three counties in Tennessee, four counties in Kentucky, and two counties in Arkansas.

DISCUSSION

Alternaria ricini is capable of infecting the seedlings, leaves, capsules, and seed of the castor-bean. Seedling infections lead to reduced stand and vigor, leaf infections cause reduced leaf area, and capsule and seed infections result in reduced yields and provide a means of spread and overwintering for the fungus. If it ever becomes necessary for the United States to go

into large-scale production of castor beans, the Alternaria disease of the castor-bean plant may well become an extremely important economic factor. No means of control have been developed, but preliminary tests indicate that the seedling phase of the disease may be controlled to some extent by seed treatment. A search is being made for disease-resistant material, although no selections have been found to date which seem to have any appreciable resistance.

SUMMARY

A disease of the castor-bean plant in the United States is described and the causal organism was determined to be Alternaria ricini (Yoshii) Hansford. The taxonomy of the fungus is discussed. The pathogen causes a seedling disease, leaf spot, and a raceme disease. The fungus is seed-borne and has been identified on castor-bean capsules from Texas, Louisiana, Mississippi, Georgia, South Carolina, Tennessee, Kentucky, and Arkansas. No means of control have been developed but there seems to be some promise for seed treatment in controlling the seedling phase of the disease.

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PATHOGENICITY OF THE DUTCH ELM DISEASE FUNGUS

L. J. TYLER AND K. G. PARKER¹

(Accepted for publication December 4, 1944)

Swingle² first reported that monoconidial and monoascosporic progenies of Ceratostomella ulmi (Schwarz) Buisman differed widely in cultural characters when grown on nutrient agar. An intensive study by Walter,3 of variants that originated as sectors in mass isolates from diseased elms and as sectors in monoconidial progenies, revealed the existence of numerous cultural races in the same fungus. He ascribed the development of these cultural races to mutation. Since C. ulmi is normally heterothallic, 2, 4 Walter emphasized that such races might arise also through the sexual process. Observations by the present writers over a period of 8 years on cultures of monoconidial progenies from sector variants that appeared in mass isolates of C. ulmi generally confirm those of Walter.

The existence of races, within the species Ceratostomella ulmi, that differ in cultural characters suggested that they might differ also in pathogenicity to the elm. In 1932, Buisman⁵ stated that lines of C. ulmi differ in virulence but the extent and constancy of these differences was not revealed. paper is to present some data concerning significant differences noted in the capacity of different races to produce disease and data which further show that each race was constant in this character.

MATERIALS AND METHODS

Fungus. Eight cultural races of Ceratostomella ulmi were used: they originated from diseased elm tissue collected in the summer and fall of 1934. Races A1, A2, A3, and A4 each arose as a sector variant in a culture of C. ulmi obtained from diseased tissue taken from a tree at Elmsford, New York. Races B, C, D, and E orginated similarly but from different diseased trees; the latter trees were in Bronx borough of New York City. Stock cultures of the different races were started, during the winter of 1935, from single spores except for race E. This last race was entirely mycelial in type of growth and did not sporulate, so the stock culture was started from a single hypha. In addition to studying the pathogenicity of each race separately, seven of them were combined and used as a "mixture." Stock cultures of the different races were maintained on potato-dextrose agar and kept at ordi-

son Institute for Plant Research, Yonkers, New York, is gratefully acknowledged.

² Swingle, R. U. A preliminary note on sexuality in the fungus *Ceratostomella ulmi*. Phytopath. 26: 925-927. 1936.

4 Buisman, Christine. Ceratostomella ulmi, de geschlachtelijke vorm van Graphium ulmi, Schwarz. Tijdschr. Plantenz. 38: 1-5. 1932.

5 Buisman, Christine. Verslag van de phytopathologische onderzoekingen over de iepenziekte, verricht in het Laboratorium, Willie Commelin Scholten, gedurende 1931. Tijdschr. Plantenz. 38: 17-36. 1932.

¹ The use of laboratory facilities and field space made available by the Boyce Thomp-

³ Walter, J. M. Variation in mass isolates and monoconidium progenies of Ceratostomella ulmi. Jour. Agr. Res. [U. S.] 54: 509-523. 1937.

nary room temperature until the end of the year 1940. Afterward they were stored at about 5 $^{\circ}$ C.

Inoculum was developed by growing the fungus in Petri dishes on sterile, cellulose, beer-glass coasters soaked with potato-dextrose solution. Spores formed on this medium were readied for use as inoculum by suspending them in sterilized distilled water. The density of the spore suspension of each race was adjusted to about ½ million spores per ml. Since race E did not sporulate, the mycelium was used as inoculum without suspending it in water.

Trees. American elms (Ulmus americana L.) were grown from cuttings obtained from a single source and rooted during June, 1938. In April, 1940, the young trees were transplanted to the field. The planting of elms comprised 22 rows of 12 trees each and was located on a valley bottom in the Boyce Thompson Research Institute Arboretum at Yonkers, New York. At the time of inoculation the trees were in their 5th year of growth and from 6 to 9 feet high. There was considerable variation in vigor but this was offset by randomization; all trees were in an active stage of shoot elongation when they were inoculated.

Inoculation. The inoculum was introduced into the trees with a 10-ml. hypodermic syringe equipped with a 11/16-inch, No. LN special Becton-Dickinson & Co. needle which has the delivery aperture on its side near the point. After loading the syringe with the spore suspension the needle was inserted underneath the bark, on a tangent with the trunk circumference, at one point on each of two sides but at slightly different levels and about 2 feet above the soil line. As the needle was forced underneath the bark care was taken to injure some peripheral wood vessels to assure ingress. Three ml. of inoculum were delivered at each point. Since race E did not produce spores, bits of mycelium were introduced into small longitudinal trunk incisions that extended into the outermost vessels; after the inoculum was introduced into the wound the latter was covered with waterproof tape. The work of inoculating the trees was completed within 3 hours on June 19, 1942.

Eleven trees were utilized for each of the cultural races A2, A3, A4, B, C, D, and E, while for race A1 and for the "mixture" the number was twenty-two. Trees inoculated with a given race occurred only once within a row, and the different races were well randomized with respect to each other.

Recording data. The approximate percentage of foliage wilted per tree was estimated at intervals of 11, 20, 34, and 73 days following inoculation. The amount of dead wood per tree was accurately determined on October 21–22, 1942, in the following manner: the length of the trunk and of all branches of each tree up to the end of the 1941 growth was measured, then the linear inches of dead wood were obtained by measuring downward from the point at which 1941 growth had ceased. The significance of the differences between the mean amounts of dead wood per tree caused by the different races was analyzed statistically.

In May, 1943, the number of trees killed by the different races was recorded and the trees were cut and examined for discoloration. All of the cultural races used for inoculative purposes were recovered by making tissue cultures.

RESULTS AND DISCUSSION

Examination of the means for dead wood (Table 1) shows that cultural races A3, A4, and C are significantly weaker pathogens than the others. Furthermore, race A4 is less virulent than A3 and both are significantly less pathogenic than A1 and A2. It is recalled that races A1, A2, A3, and A4 originated more or less simultaneously as sector variants in a culture

TABLE 1.—Pathogenicity of different cultural races of Ceratostomella ulmi to the American elm

Cultural race -	day	Index of safter			$_{ m trees\ killed^b}$	Mean per cent of dead wood per tree		
1200	11	20	34	73	trees kineu	dead wood per trees		
A1	73	88	95	97	22/18	93.0		
A2	74	93	92	89	11/5	86.2		
A3	7	45	49	50	11/0	43.6*		
A4	0	26	35	26	11/0	22.8*		
В	64	94	91	89	11/6	83.0		
C	0	36	51	46	11/0	34.2*		
D	56	85	88	93	11/9	89.2		
Eq	0	0	0	0	11/0	None		
Mixture	67	91	91	92	22/14	91.2		

a Total possible wilt = 100.

b Numerator = number of trees inoculated; denominator = number killed.

d Data were not used in the statistical analysis.

obtained from one diseased elm. Since A3 and A4 were less pathogenic than A1 and A2 it is evident that some loss in pathogenicity accompanied the appearance of sector variants from which the former 2 races originated. It is assumed that A1 and A2 were like the parent culture which must have been strongly pathogenic since the tree from which it was obtained died rapidly. The nonsporulating race E proved to be nonpathogenic; this organism never spread (as shown by discoloration in the elm tissue) more than 2 to 6 inches away from the inoculation points. This is a striking contrast to the invasiveness of the others. The strongly pathogenic races (A1, A2, B, and D) produced heavy discoloration of the tissue throughout the 1942 annual ring of the branches and trunks, while that caused by the moderately pathogenic races (A3, A4, and C) was equally intense but not so uniformly and widely distributed. The downward spread of the weaker races in the trunks was definitely limited and this was correlated with a general absence of dead wood in the trunks. The composite culture listed as "mixture" was equally as virulent as the most strongly pathogenic races and so it appears that the association of the weaker races with the stronger exerted no inhibitive effect upon the invasive activities of the latter.

[°] M.S.D. at 5 pct. point: 18.2 between groups with 11 trees each, 12.9 between groups with 22 trees each, 15.8 between groups with 11 trees and those with 22. Mean numbers with an asterisk are significantly different from others.

The data concerning wilt and actual number of trees killed by the different cultural races (Table 1) are self-explanatory. These data further help to emphasize the marked differences in pathogenicity between some of the races.

The pathogenic behavior of the cultural races studied suggests that within the species *Ceratostomella ulmi* there exist a number of entities which possess unequal ability to produce disease. Some of these entities are strongly pathogenic, others moderately so, and still others are practically nonpathogenic. On this basis cultural races A1, A2, B, and D are strong pathogens, races A3, A4, and C are moderately strong, and race E is nonpathogenic.

Since cultural race E is a nonsporulating organism and is practically nonpathogenic it would be difficult to prove even by attempting to mate it with known male and female strains of *Ceratostomella ulmi* that it belongs to this species. However, it arose as a distinct sector variant in a culture of a typical mass isolate, and the isolate was obtained from discolored wood of an elm destructively affected by *C. ulmi*. Except for lack of sporulating structures this race looks much like other pathogenic ones when grown on nutrient agar.

The constancy of pathogenicity in the different races studied is attested to by some results obtained during the early spring and summer of 1935, seven years earlier. At that time potted elms were inoculated with all these cultural races, except A3. With allowances for the fact that but 2 to 3 trees were inoculated with a given race at that time, the results show that the pathogenicity of each race on the earlier date was essentially similar to that several years later. Races A1, A2, B, and D were strongly pathogenic, A4 and C were moderately strong, and race E was nonpathogenic. The fact that the pathogenic capabilities of the different races remained rather constant is striking and more so since all the races were continuously maintained on nutrient agar in test tubes during the 7-year period between the two tests. Race A1, only, had been passed through the elm in the interim.

Concluding, it appears that races unequal in pathogenic capabilities arose as sector variants in mass isolates of *Ceratostomella ulmi*. While these sector variants may have originated as a result of mutation, the manner of their origin needs further study.

Apart from the importance that such variants play in the diagnosis of specimens from suspected cases of Dutch elm disease,⁵ these pathogenically distinct entities might have some influence on the production of disease epiphytotics. Furthermore, this racial specialization should be considered in a disease control program involving the development of disease resistant elms.

SUMMARY

1. Culturally distinct races of *Ceratostomella ulmi* originated as sector variants in mass isolates obtained from native diseased American elm.

5 See footnote 3.

- 2. These cultural races were of unequal pathogenicity. Some of them were strongly pathogenic, others were moderately strong, and one was practically nonpathogenic.
- 3. The cultivation of these races on nutrient agar for 7 years, without passing them through the elm, did not appreciably change their pathogenicity.

DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY, ITHACA, NEW YORK.

1945]

REPORT OF THE 36TH ANNUAL MEETING AND WAR CONFER-ENCE OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 36th annual meeting and war conference of The American Phytopathological Society was held at the Netherland Plaza, Cincinnati, Ohio, December 9-11, 1944. Eighty society was need at the Netherland Plaza, Uncinnan, Onio, December 9-11, 1944. Eighty papers on original research were accepted by the editorial committee for presentation at the meeting. Approximately 300 members attended. Twenty-three papers accepted were concerned with "fungicides." Sixteen papers were placed in the section on "virus and bacterial diseases"; a section on "disease resistance" included seven papers; while six papers were included in the section entitled "seed and soil treatments." Fourteen papers dealt with "various factors affecting disease dealerment." and fourteen papers dealt with "various factors affecting disease development," and fourteen papers were in the section entitled "fungus diseases."

Conferences held were characterized by open and frank discussion of the problems considered. These conferences included "New developments in fungicides," "Future of plant disease surveys," "Seed treatments," "Breeding for plant disease resistance," "Activities of the war committee," and "Phloem necrosis of elms." The Phytopathologists' dinner was held Sunday evening, December 10.

Reports indicated that the 1944 annual meeting and way conference afforded an excel-

Reports indicated that the 1944 annual meeting and war conference afforded an excellent opportunity for members to discuss mutual problems. It also afforded an opportunity for members to exchange viewpoints and ideas that should be helpful in continuing and expanding research, teaching, and extension in Plant Pathology.

Council for 1945:

H. B. Humphrey, President (1 yr.), Box 14, Cosmos Club, Washington 5, D. C. J. H. Craigle, Vice-President (1 yr.), Central Experimental Farm, Ottawa, Canada. E. M. Johnson, Secretary (3 yr. term expires 1947), Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.

R. M. CALDWELL, Treasurer, and Business Manager of Phytopathology (3 yr. term expires 1946), Purdue University, Lafayette, Indiana.

Helen Hart, Editor-in-Chief, Phytopathology (term expires 1945), University Farm, C. H. ARNDT, Agricultural Experiment Station, Clemson College, South Carolina.

H. P. Barss, Office of Experiment Stations, United States Department of Agricul-

O. C. Boyd, Massachusetts State College, Amherst, Massachusetts. J. J. Christensen, University Farm, St. Paul 8, Minnessota.

R. W. Goss, College of Agriculture, Lincoln 1, Nebraska.
R. S. Kirby, Pennsylvania State College, State College, Pennsylvania.

L. D. Leach, University Farm, Davis, California.

Representatives:

A.A.A.S. Council. J. G. LEACH, J. C. WALKER. Division of Biology and Agriculture, National Research Council. J. C. Walker. Board of Editors, American Journal of Botany. A. A. Dunlap.

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Regulatory Work and Foreign Plant Disease. C. R. Orton, R. P. WHITE, E. C.

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Union of American Biological Societies (and Biological Abstracts). Donald Folsom, L. M. Massey, W. C. Snyder, W. G. Stover, F. V. Rand, Chm.; Helen Hart and E. M. Johnson (ex officio).

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Coordination in Cereal and Vegetable Seed Treatment Research. C. H. Arnot, F. J. GREANEY, C. M. HAENSELER, K. W. KREITLOW, L. D. LEACH, R. W. LEUKEL, J. H. McLaughlin, George Semeniuk, M. B. Moore, Chin.

Fungus Nomenclature. C. M. Tucker, D. S. Welch, Erdman West, G. L. Zundel, J. A. Stevenson, Chm.

Nomenclature and Classification of Plant Viruses. C. W. Bennett, L. M. Black, James Johnson, H. H. McKinney, Frank McWhorter, H. Earl Thomas, Freeman Weiss, Chm.

Plant Disease Prevention. J. F. Adams, K. D. Butler, C. E. F. Guterman, E. C.

STAKMAN, R. P. WHITE, C. G. WOODBURY, DONALD FLETCHER, Chm. Publication Problems. M. W. GARDNER, FRANCIS O. HOLMES, A. J. RIKER, Chm.;

R. M. CALDWELL and HELEN HART (ex officio).

Reorganization of International Cooperation. H. P. Barss, G. H. Coons, J. G. Har-Rar, Otto Reinking, J. A. Stevenson, E. C. Stakman, Chm. Standardization of Fungicidal Tests. M. C. Goldsworthy, C. S. Holton, J. G. Hors-Fall, M. B. Moore, C. F. Taylor, H. W. Thurston, J. D. Wilson, S. E. A. Mc-CALLAN, Chm.

Terminology (Nomenclature) of Immunology and Use of Technical Words. D. L. Bailey, W. H. Burkholder, Donald Folsom, M. W. Gardner, Chm. War Committee. J. G. Leach, E. C. Stakman, I. E. Melhus, Chm. (Executive

Committee).

Temporary Committees for 1944:

Auditing. C. T. Gregory, George B. Cummins, Chm.

Illustrative Material for Papers. E. D. Hansing, G. C. Kent, E. J. Anderson, Chm.

Publication of Membership List. R. M. Caldwell, Helen Hart, J. G. Leach, R. S. KIRBY, Chm.

Resolutions. R. W. Goss, F. L. Howard, C. M. Tucker, Chm.

Temporary Committee for 1945:

Rec

Publication of Special Material. H. P. Barss, J. G. Leach, W. H. Tisdale, W. G. STOVER, Chm.

Report of the Secretary. At the time of our Council Meeting, December 6, 1943, the membership was 1,060. It now totals (December 11, 1944) 1,089. This makes a net gain of 29 members. During the period from December 6, 1943, to December 11, 1944, 63 individuals applied for membership.

Twenty-nine former members have been reinstated, and the Society lost a total of 63

members: 16 by resignation, 9 by death, and 39 suspended for nonpayment of dues.

memoers: 10 by resignation, 9 by death, and 39 suspended for nonpayment of dues. On January 2, 1944, a letter, one copy of the original application, the confidential slip, and new application blanks were sent to all members with applications in the Clearing Agency. Each applicant was asked to bring his application up-to-date on the new application forms and confidential slip enclosed and return to the Secretary's office. If no change was necessary, the applicant was asked to return the original copy of the application up-to-date on the proposed and return the original copy of the application up-to-date on the proposed and return the original copy of the application up-to-date on the proposed and return the original copy of the application up-to-date on the proposed and return the original copy of the application up-to-date on the proposed and return the original copy of the application up-to-date on the new app tion and original confidential slip.

Applications of those who did Not return revised, or original application were removed from the ACTIVE file of the Clearing Agency. The number removed totaled 37; 20 were placed on the INACTIVE list by request, and 12 for nonpayment of dues for 1943. There are now 35 applications in the ACTIVE file of the Agency—of these 9 are new.

During the year 66 applications were sent to 20 employers.

Report of the Treasurer. Statement of accounts for the year ending September 30, 1944.

Balance from 1	943			 	 	 					\$2296.28
Annual dues:		100		 	 	 	\$	14.00			
1944					 	 	2	624.24			
1945		••••••				 		43.44	\$26	81.68	
30-Year-Index					 	 				11.00	
Check for colle				 	 	 				4.95	
Sales		************								1.80	
Total receipts			**************	 	 	 					2699.4

Member subscriptions transferred to PHYTOPATHOLOGY: 1943 \$ 12.00 1944 2100.50 1945 28.50 \$2141.4	
1944	
1045	00
	JU
Transferred to PHYTOPATHOLOGY for:	
Sales 1.80 30-Year-Index 11.00 12.	00
Secretarial work and expenses of Office of Secretary 173. Secretarial work for Treasurer 235.	
100000000000000000000000000000000000000	
	50
Exchange charge	
Telegrams 2.	
Travel expense in moving society offices, Beltsville, Maryland to	20
Lafayette, Indiana 89.	80
Data y colo, intrata	
Fotal expenditures	\$2979.37
Balance on hand	2016.34
	\$4995.7]
	*
Sinking Fund. There has been no change in the principle amount of the s	
luring the past year, the total remaining at \$9676.00. It is invested as follow	
First mortgage note deposited with McLachlen Banking Corporation fo	\mathbf{r}
collection (\$500.00 at 4½%)	\$ 500.00
United States Savings Bond, Series G. 21%	1000.00
Invested with the following:	
Arlington and Fairfax Building and Loan Association, 4%	1000.00
Columbia Permanent Building Association, 4% (accrued interes	it
\$35.92)	535.99
District Building and Loan Association, 3½% (accrued interes	t
\$103.83)	1603.83
National Permanent Building Association, 4% (accrued interes	it
\$175.45)	2175.49
Northwestern Federal Savings and Loan Association, 3\frac{1}{2}\%	2000.0
Perpetual Building Association, 4% (accrued interest \$71.84)	1071.8
Prudential Building Association, 31% (accrued interest \$20.47)	196.4
Less interest due PHYTOPATHOLOGY	\$10083.5
Less interest due PHYTOPATHOLOGY	407.5
	\$ 9676.00
Who Termon Managed The 3 77 7 0	
The Lyman Memorial Fund, obtained from voluntary contributions,	now total
$\sharp 3175.32$. The whole amount is invested with the Brookland Building and eiation, at $3\frac{1}{2}\%$. The account for 1944 follows:	Loan Asso
elation, at 32%. The account for 1944 follows:	
Balance on hand, December 1, 1943	\$3285.8
Dividends, December 31, 1943, to June 30, 1944	99.0
	\$3384.8
Township and a programme of the state of the	
Less interest due PHYTOPATHOLOGY	209.5
Less interest due PHYTOPATHOLOGY	
Less interest due PHYTOPATHOLOGY	
Additional Endowment:	
Additional Endowment: War Savings Bonds, series F	\$3175.3
Additional Endowment: War Savings Bonds, series F Received prior to December 1, 1943	\$3175.3
Additional Endowment: War Savings Bonds, series F Received prior to December 1, 1943 Received December 1, 1943, to September 30, 1944	\$3175.3
Additional Endowment: War Savings Bonds, series F Received prior to December 1, 1943 Received December 1, 1943, to September 30, 1944 War Savings Stamps and Cach	\$3175.3 \$3175.3 \$ 725.0 325.0
Additional Endowment: War Savings Bonds, series F Received prior to December 1, 1943 Received December 1, 1943, to September 30, 1944 War Savings Stamps and Cash Received prior to December 1, 1943	\$3175.3 \$725.0 325.0
Additional Endowment: War Savings Bonds, series F Received prior to December 1, 1943 Received December 1, 1943, to September 30, 1944	\$3175.3 \$725.0 325.0
Additional Endowment: War Savings Bonds, series F Received prior to December 1, 1943 Received December 1, 1943, to September 30, 1944 War Savings Stamps and Cash Received prior to December 1, 1943	\$3175.3 \$3175.3 \$725.0 325.0

Report of the Business Manager. The total number of nonmember subscribers was 483 on November 30, 1944, representing a net gain of 52 for the year, 1944. These consisted of 300 domestic, 32 Canadian, and 151 foreign subscribers. Included in the domestic subscriptions for later foreign shipment are 20 for the Chinese Ministry, 31 for the USSR, and 7 for the Netherlands; also 5 complimentary domestic subscriptions. There were 47 subscription cancellations and suspensions in 1944. Not included in the reported subscriptions for 1944 are 55 subscriptions for the current volume, number 34, by the American Library Association, to be held by the Society for eventual shipment to foreign countries as may be designated by the Library Association.

The sales of back volumes and issues during 1944, included in addition to smaller items amounts of: \$576.00 to the Chinese Ministry of Education; \$184.00 to the French Colonial

Supply Mission; and \$135.00 to the American Library Association.

Statement of accounts for the year ending September 30, 1944.

Balance from 1943			\$ 6750.2
Subscriptions			
1943	\$ 18.00		
1944	2548.70		
1945	288.95		
1946	116.00	\$2971.65	
Member subscriptions:			
1943	12.00		
1944	2100.50		
1945		2141.00	
Sales of back numbers of PHYTOPATHOLOGY		1601.94	
Advertising			
1943	68.18		
1944	844.70		
Advertisement for membership list	50.00	962.88	
30-Year Index		68,00	
Total and the Circles of Them 3			
First mortgages	. 21.50		
Building and Loan	100.00		
U. S. Bond Series G	25.00	146.50	
Interest on current funds		140.81	
Grant from Rockefeller Institute		600.00	
Allowance on reprints		533.27	
From authors for excess illustrations		271.05	
Smith Memoirs			
Refund of overpayment			
Total receipts			9602.

\$16352.56

Expenditures:

Printing, distributing, and storing PHYTOPATHOLOGY:	
Vol. 33, no. 11	
12 1026.34	
Vol. 34, no. 1	
2	
3 783.31	
4	
5	
6 712.39	
712.11	
8	
	\$8720.96
Secretarial work and office expense, Editor-in-chief	348.60
Secretarial work for Advertising Manager	111.75
Postage for Advertising Manager	9.00
Commission for Advertising Wanager (7042)	100.00
Commission for Advertising Manager (1943)	
Other expense (telephone and printing) Advertising Manager	9.68
Secretarial work for Business Manager	314.01
Office supplies	12.17
Express in transferring office equipment and records, Beltsville,	
Maryland, to Lafayette, Indiana	61.00

Claren v	2.50	
Stamps Printing Recondition typewriter	81.02	
Recondition typewriter	15.00	
Postage, 30-Year Index (part)	9.10	
Refund subscription	5.17	
Overcharge, Science Press	164.67	
Purchase of back numbers	14.00	
Bank charge	1.38	
Total expenditures Balance on hand:		\$ 9980.03
Checking account Northwestern Federal Savings and Loan	\$1572.86 4799.69	6372.55
		\$16352.56
The 30-Year Index. Summary of receipts and expenditures Nov	ember, 19	
		43, to Sep \$ 59.58
mber 30, 1944: Balance in excess of expenditures Nov., 1943 Receipts Nov., 1943, to Sept. 30, 1944		\$ 59.58 \$ 59.58 68.00 \$127.58
mber 30, 1944: Balance in excess of expenditures Nov., 1943		\$ 59.58 \$ 59.58 68.00 \$127.58
mber 30, 1944: Balance in excess of expenditures Nov., 1943 Receipts Nov., 1943, to Sept. 30, 1944		43, to Sep 59.58 68.00 \$127.58 9.10

Report of the Auditing Committee, as of September 30, 1944. We have examined the books of the Treasurer of the American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY and find them to be correct.

Signed: GEORGE B. CUMMINS, Chairman CHAS. GREGORY

Report of the Advertising Manager for 1944. This report covers the calendar year, January 1 to December 31, 1944. During this period 25 companies inserted a total of 122 revenue-producing advertisements in the regular journal and in the membership list. These insertions occupied 85 pages and consisted of 68 full-pages, 37 half-pages, and 17 quarter-pages. Nonrevenue-producing advertisements totalled II and occupied 41 pages involving 1 full-page, 4 half-page, and 6 quarter-page insertions relative to Phytopathological Classics, The Society Clearing Agency, 30-Year Index, Phytopathology Endowment Fund, and Biographical Memoirs of Erwin F. Smith.

Gross receipts from advertising totalled \$1,685.00. The net profit to the Society after costs of printing and office expenses connected with the advertising, and agency

commissions and discounts was approximately \$748.00.

With the approval of the Council of the American Phytopathological Society, advertising was included in the 1944 issue of the membership list. It was largely through the efforts of the membership list committee, and J. C. Walker that enough revenue was collected from the advertising carried in the list to leave a small profit above the cost of publication.

Report of the Editor-in-Chief. There are 1085 pages, exclusive of the index, in volume 34 of PHYTOPATHOLOGY. The volume contains 106 articles, 36 notes, 3 reports time 34 of FHTTOFATHOLOGY. The volume contains 100 articles, 50 notes, 5 reports of meetings, 118 abstracts, 5 biographies with portraits, 5 book reviews, 4 notices or announcements, and 224 text figures. A membership list, of 36 pages, forms a supplement to the September issue. The present editor-in-chief was appointed by the Council on March 1, to fill the unexpired term of H. B. Humphrey. Since January 1, 1944, eight papers have been rejected or withdrawn. On November 15, 1944, there were 76 papers are band. 42 have been accorded for publication 10 and hour provined by the outbook and on hand: 42 have been accepted for publication, 10 are being revised by the authors, and 24 are under consideration by the editorial board. Appraisals and criticisms are secured from two, three, or four members of the editorial board before a paper is accepted for publication.

With the approval of the Council, and for a short time, PHYTOPATHOLOGY has extended the courtesy of its pages to foreign scientists who are persevering in research under difficult war-time conditions and are deprived temporarily of opportunity for

membership in the American Phytopathological Society.

The editorial board will be helped greatly and the necessity for conserving paper may be met, if authors will recognize the need for, and possibilities of, condensation. An author's condensation usually is far more satisfactory than an editor's. Tables often

can be simplified, presented in summary form, or even eliminated and replaced by a short paragraph. Illustrations may not be essential to a paper, many that are submitted cannot be reproduced well, and some do not justify the space they require. Better planning of tables and illustrations for the journal dimensions also will facilitate the work of both editorial board and printer. Conservation of space by judicious condensation will enable a greater number of members to publish in the journal.

It is gratfying to find the authors as cooperative and patient as they have been this year when it is difficult to secure adequate editorial assistance and to maintain normal publication procedures. Members of the editorial board have shared the responsibilities of the editorial office and have given invaluable assistance in the appraisal and criticism

of manuscripts.

Report of Society Representative on the National Research Council for 1944. Your representative attended the Annual Meeting of the Division of Biology and Agriculture at Washington, D. C., in April, 1944. Actions and discussions of interest to the Society are as follows:

1. It was recommended to the Council that an Agricultural Board be established to which general problems relating to Agriculture can be referred. This board was subsequently created as follows: W. C. Coffey (Chairman), C. H. Bailey (Vice Chairman), R. E. Buchanan (Secretary), E. C. Auchter, T. P. Cooper, C. B. Hutchinson, W. H. Martin, L. A. Maynard, H. P. Rusk, R. F. Griggs (ex-officio), Ross G. Harrison (ex-officio). At its first meeting at Minneapolis (September 25–26, 1944) the Board undertook to sponsor the present committees of the Division which are related to Agriculture. This would include the Committee of the Division which are related to Agriculture. include the Committee on Crop Protection (E. C. Stakman, Chairman).
2. The establishment of a subcommittee on National Research Council Fellowships

in Agriculture was recommended with the idea that funds be sought to establish for the norther war period a series of Fellowships in Agriculture distinct from those now available in the Natural Sciences. This Committee has been authorized by the Council and will work in association with the Fellowship Board.

3. The Division voted to set up a Committee on Personnel Problems to consider the problems that will be faced by scientists in various fields upon demobilization of the armed forces. It was pointed out that such a committee should work with, rather than replace, systems already in existence within certain Societies (such as that in the American Phytopathological Society).

4. The question of prepublication of abstracts was discussed without any final action.

Report of the Representative to the Board of Editors, American Journal of Botany. One paper, on localization of wilt resistance in tomato, submitted for publication in the American Journal of Botany was reviewed and comments furnished to the editor-in-chief. An invitation to read a paper on certain genetic studies with an ascomycete was declined since it appeared to be somewhat out of the field of plant pathology.

As a suggestion, the question might be asked: Has the Phytopathological Society ever considered the advisability of associating with its Editorial Board representatives from certain other societies in closely related fields?

Report of Committee on Donations and Legacies. One appeal went out to the entire membership to purchase war bonds and stamps, or make contributions to The American Phytopathological Society for the purchase of bonds and stamps, to enhance the Lyman Memorial Fund. The response was almost negligible, since during the entire fiscal year only one contribution had been made from an individual in the form of a Series F Bond with a maturity value of \$25.00. In addition, the Pacific Coast Division of The American Phytopathological Society had contributed three Series F Bonds each with a maturity value of \$100.00.

Your Committee has had considerable correspondence relative to the possible reasons why our membership did not respond to these appeals over the past two years in a more generous fashion than they did. Very evidently, the cost of the appeals to the member-

ship did not bring in results commensurate therewith.

It would be the chairman's recommendation that the Committee be completely reorganized with new personnel which will bring new viewpoints and new ideas to it. It would also be the chairman's recommendation that Mr. N. J. Giddings, who is responsible for the Pacific Coast Division contributions, be made the permanent chairman of this Committee. During the past two years he has shown a high degree of interest in the Committee's activities and will ably continue its work.

J. G. Brown, N. J. Giddings, B. L. Richards, N. E. Stevens, R. P. White, Chairman

Report of the Extension Work and Relations Committee. A kodachrome transparency exchange has been instituted and has resulted in the distribution of 3,818 slides on

Receipts:

\$484.78

vegetable diseases, 1,197 slides on fruit diseases. In 1944 a set of cereal disease transparencies was collected and an opportunity presented to most Plant Pathologists to select and secure copies for their own educational work. It is hoped that this visual educational material will result in better farmer education in disease control.

A manual of extension methods in Plant Pathology was prepared by a group of pathologists under the supervision of Dr. Haskell and distributed to Plant Pathologists and heads of Extension work throughout the United States and Canada. During the past year further distribution to workers in foreign countries has continued on a request basis. The exchange of pertinent control articles, mimeographs and printed matter, has continued in an attempt to bring the new work to the attention of those interested.

A movement has been started to assemble pictures, diagrams, and drawings of disease specimens for exchange (much as the transparencies have been). The sub-committee feels the need of better illustrations in pathological control literature and would welcome suggestions as to (1) a method of securing adequate illustrative material, and (2) a means of making this material available for State use.

Report of the Necrology Committee. Deaths of members during 1943 not previously

JAMES MCMURPHY (date not known) G. W. Goldsmith, August 28

Deaths of members during 1944:

DEAN B. SWINGLE, January 8 P. H. Rolfs, February 23 W. T. Horne, April 12 A. H. R. BULLER, July 3 E. F. GAINES, August 17 ERNEST DOPP, September 24 H. H. WHETZEL, November 30

Report of the Manager of Phytopathological Classics. beginning December 1, 1943, and ending September 30, 1944: Report for the fiscal year

Classics No. 1: On hand Dec. 1, 1943 Sold during year		
On hand Sept. 30, 1944 Classics No. 2: On hand Dec. 1, 1943 Sold during year		15
Classics No. 3: On hand Sept. 30, 1944 Sold during year		216
Classics No. 4: On hand Sept. 30, 1944 On hand Dec. 1, 1943 Sold during year	374	306
On hand Sept. 30, 1944. Classics No. 5: On hand Dec. 1, 1943 Sold during year		366
Classics No. 6: On hand Dec. 1, 1943 Extras found Sold during year	696 7	604
On hand Sept. 30, 1944 Classics No. 7: On hand Dec. 1, 1943 Extras found Sold during year	718	692
On hand Sept. 30, 1944 Cash balance on hand Dec. 1, 1943 Receipts during year		716 \$442,28
Total		42.50

Tranen ditarres .

Postage Stationery	\$ 5.75 16.00	
Total expenditures		\$ 21.75
Balance on hand Sept. 30, 1944	•••••••	\$463.03 21.50

Report of the Public Relations Committee for 1944. Slight changes in policy, with a view of greater decentralization of responsibility and more evenly divided territorial spread have been applied through the committee membership with varying success. Probably further changes would be beneficial, particularly in regard to including in unit areas

ably further changes would be beneficial, particularly in regard to including in unit areas lands geographically contiguous and producing similar crops.

The responsibility and actual work of the committee, previously largely carried by the chairman, has been passed on to the committeemen in their respective geographical areas largely because of the diversity of crops and likewise the wide range of application of the technical material contained in PHYTOPATHOLOGY throughout the year.

The scope of the work has been slightly enlarged and the reception probably better. Local spreads in various localities in daily newspapers, Sunday supplements, and farm magazines have been spasmodic but increasingly impressive. For instance, several pages of plant diseases and fungi in colors have appeared in different magazines of national

distribution and scope during the past year.

The general response of the society members as to their opinions and reactions to this activity appears definitely to be increasingly favorable and voluminous. The necessity and importance of this work in relation to the general welfare of the society and its members as individuals have frequently been manifested. In the past, we as a group have members as individuals have frequently been manifested. In the past, we as a group have probably suffered less rapid progress because of our reluctance to devote at least a small portion of our time and energy explaining to the public the scope of our work and its application. It can hardly be denied that any business successfully conducted devotes certain of its energies informing the people. It is the general belief of the committee that the field and scope of phytopathology has been more successfully interpreted and understandingly placed before the general public than any year of the past in spite of the continued competition in publicity due to the war.

Some changes in personnel have been made and others should be made whenever any member is found with ability to popularize our technical findings.

Report of the Committee on Regulatory Work and Foreign Plant Diseases. The standing committee on Regulatory Work and Foreign Plant Diseases, consisting of C. R. Orton, R. P. White, and E. C. Stakman, chairman, has little to report. Dr. White was acting chairman while the chairman was in Mexico during the spring of 1944.

There are now in reality three committees or subcommittees, representing more or

less directly the Society, that are concerned with quarantines and regulatory work:

- 1. The subcommittee of the War Committee, known as the plant quarantine subcommittee
- 2. The Crop Protective Committee of the National Research Council

3. The standing committee

The activities of committee No. 1 will be summarized in the report of the War Committee. Committee No. 2 was authorized at the Columbus meeting to represent the interests of the Society in matters affecting entomology and plant pathology jointly. Committee No. 3 was authorized at the Columbus meeting, and its function was restricted to giving advice, when called upon, to the federal Bureau of Entomology and Plant Quarantine.

Committee No. 2 concentrated on an attempt to get the U.N.B.R.A. to take all feasible precautions in preventing the promiscuous dissemination of insect pests and plant patho-

gens. In spite of the fact that the committee actually put forth considerable effort, the results were somewhat disappointing, although the officials of this organization probably have consulted more freely with the federal Bureau of Entomology and Plant Pathology than might otherwise have been the case. Perhaps, after all, the committee could not go very much farther than to influence the U.N.R.R.A. organization to avail themselves of the help and advice of official organizations charged with the responsibility of safe-

guarding against the introduction of new or especially dangerous pests and pathogens.

As concerns the activities of Committee No. 3 specifically, the federal Bureau of Entomology and Plant Quarantine furnished information regarding the important plant diseases taken in connection with insect and plant disease surveys in the general vicinity of ports of entry from June, 1943, to December 31, 1943, and consulted with the committee regarding the potato wart situation in the United States. Inasmuch as committees 1 and 2 concerned themselves with attempting to get proper action, committee No. 3 restricted its function to its mandate, namely, to give advice when the advice was sought.

Report of the Committee on Biological Abstracts and the Union of Biological In general, the present picture for BIOLOGICAL ABSTRACTS is a good one: The enterprise is holding its own and in some respects improving and advancing in spite of the continuing difficulties of the hour. As with most current activities—governmental and private—this is the critical moment to continue full speed ahead and at the

same time to plan for the post-war period.

Abstracts of Plant Sciences. The complete volume of Biological Abstracts for 1944 will contain 23,314 abstracts as compared with 25,947 in the preceding year; Secton D-Abstracts of Plant Sciences will contain 5,701 this year as compared with 6,516 in 1943. Abstracts of Plant Sciences will contain 3,701 this year as compared with 6,010 in 1943. This section includes: Ecology, systematic botany, morphology and anatomy of vascular plants, agronomy, horticulture, forestry, pharmacognosy and pharmaceutical botany, plant physiology, and phytopathology. For the year it contains 1,277 abstracts of contributions dealing directly or indirectly with plant diseases, three-fourths of which are in the division devoted to plant pathology so ably edited by Dr. Freeman Weiss.

Current output of published research. One feature of the times is that a large amount of the contribution of the

of scientific activity still going into research cannot-for military reasons-be published now. Furthermore, many of the active scientists of the warring nations are in the armed forces or in one way or another serving their governments in capacities precluding further current research or the publication of that already completed; this situation also renders many of those who formerly cooperated with Biological Abstracts unavailable for abstracting or for other essential services to the journal. The research literature available is therefore somewhat smaller than in recent years, yet Biological Abstracts continues to abstract all that is obtainable promptly and with unimpaired scholarship. At the present time about 2,000 journals are being covered, and cooperation with other American and British abstracting services is continuing.

Efforts directed toward the procurement of European journals for abstracting have gone on vigorously in spite of difficulties which all will recognize. More recently, about 170-mainly German and medical-are available for this purpose and are being abstracted as rapidly as possible. Active efforts are also being made to procure and abstract the literature of Russian research. For a number of years only a small trickle of this material has been obtainable, but recent weeks have seen an encouraging increase

Post-war publication. In the months that will follow the conclusion of the war in Europe, Biological Abstracts will undoubtedly face one of its greatest tests. indications seem to be that there are at least 2,500 and possibly as many as 3,500 European publications, unavailable since the onset of the war, which should be abstracted at once. This includes the bulk of the German literature and nearly all of the French, Italian, Scandinavian, Dutch, Belgian, and Swiss. The policy of Biological Abstracts will be to procure these journals and abstract them from the beginning of the war, but stocks of such publications available for export are scant and the procurement, assignment, and abstracting of this vast amount of material are bound to present major difficulties. The Trustees and Staff of Biological Abstracts solicit the advice, assistance, and cooperation

of all to help them carry out this program promptly and in a scholarly manner.

Financial status. Thanks to a steady increase in the number of subscriptions and to the generosity of nearly 100 industrial corporations, supplementing previous channels of income, the organization expects to complete the year substantially with a balanced budget. Estimated income for 1944 will be approximately as follows: Subscriptions \$49,460.18, advertising income \$2,428.04, net from sale of back volumes \$6,010.96, and contributions from societies \$3,049, making with contributions of \$12,770 from the industries a grand total of \$73,718.68; the operating budget for the year was \$72,740. Considerable satisfaction is justifiable from the fact that Biological Abstracts is now running on budgets that are substantially in balance with income slowly growing from year to year. It should not be forgotten, however, that this situation is very largely due to the generous subsidies received from corporations in the biological field-about a sixth of the total budget. Except for their help Biological Abstracts would be forced to draw on its meager reserve and also to curtail its publication. While these corporations undoubtedly value Biological Abstracts and want to assist, it should be remembered in estimating the future stability and growth of Biological Abstracts that these contributions mostly in the form of \$100 gifts, in some cases as much as \$500-are commitments for one year only and that there is no absolute assurance that such support will continue indefinitely. Up to 80 per cent of such gifts can now be deducted in figuring income taxes. How a post-war decline in the prosperity of the corporations or a change in the tax laws might affect the present attitude is a question. Biological Abstracts cannot safely rely on contribution subsidies as a permanent basis of income; it is now therefore up to all biologists to make every effort toward increasing institutional, departmental, and personal subscriptions as a basis for adequate permanent support.

Subscriptions continue to increase slowly but steadily; the income to the current volume from this source stands about \$6,200 more than a year ago, the number of subscriptions for the complete edition having increased by 203 (98 domestic, 105 foreign) and that for the sectional subscriptions by 209 during 1944. American subscriptions to Section D (Plant Sciences) have increased from 385 to 403.

Lest we forget. To sum up the situation, Biological Abstracts has in 1944 added another creditable volume, abstracting the current literature of biology insofar as it has been available, has gone through the year substantially on a balanced budget, has materially increased its subscription income, and is looking toward the future with confidence. Year by year an instrument is being fashioned that is destined to become a progessively more important and more perfect tool for the increase, diffusion, and application of man's knowledge in the life sciences.

Biological Abstracts gratefully acknowledges the fine cooperation of its 3,000 collaborators and 150 section editors which has made its achievements possible despite the smallness of its central office staff. At the present time the Business Manager and his secretary, two central office editors (Dr. John E. Flynn, Editor in Chief, and Dr. Jean MacCreight, Assistant Editor), and two proofreaders are included in a total editorial office staff of twelve. Their accomplishment is an example of effectiveness that requires no comment and merits the appreciation of all.

Report of the Committee on Resolutions. BE IT RESOLVED THAT The Amer-

ican Phytopathological Society express its grateful appreciation to the following for their contributions to the success of its 36th annual meeting and war conference:

a. The Committee on Arrangements consisting of: W. D. Valleau, Chairman; Stephen Diachun, E. M. Johnson, A. J. Ullstrup, and O. T. Wilson for its services in providing many conveniences.

b. The management of the Netherland Plaza, and especially Miss Mary M. Hesse, Otto Hecker, and William P. Pfeiffer for their interest in making adequate facilities

c. The newspapers: Cincinnati Times-Star, Cincinnati Post, and Cincinnati Enquirer for their effective handling of news coverage.

d. The Cincinnati Chamber of Commerce for its courtesy in providing assistance in registration.

g. Mr. Harry R. O'Brien for his preparation of news releases.
f. The Ohio State University for the use of projection equipment.
g. Mrs. Jeanne Valleau Gaines, and R. R. Kincaid for adding interest and enjoy-

ment to our annual dinner.

BE IT RESOLVED THAT the Society express its gratitude to the retiring Secretary, C. C. Allison, for his efficient performance of the duties of the office during the past

three years, and to Miss Marjorie L. Jones for her capable assistance.

BE IT RESOLVED THAT the Society express its gratitude to H. B. Humphrey for his services as editor-in-chief of PHYTOPATHOLOGY from 1929 to 1944. His untiring efforts have established for the Journal a reputation as one of the outstanding publications in the field of plant science.

BE IT RESOLVED THAT the Society express its appreciation to Livia Appel, P. K. Baird, C. A. Peerenboom, J. B. Schramm, and W. A. Sumner for contributing data and advice on publication problems.

Respectfully submitted, R. W. Goss F. L. HOWARD

C. M. TUCKER, Chairman

ACTION TAKEN BY THE COUNCIL AND SOCIETY AT THE 1944 ANNUAL MEETING

Elections and Appointments. A committee, appointed by the Council, opened and counted the ballots, results of which were announced to the Society at the banquet the evening of December 10: H. B. Humphrey, President; J. H. Craigie, Vice-President; R. W. Goss, Councilor at large.

The Council recommended and the Society approved the appointment of E. M. Johnson, Secretary, for a three year term, 1945 through 1947; Paul E. Tilford, Advertising Manager of PHYTOPATHOLOGY, for a one year term; and L. C. Knorr, Editor and Acting Manager of Phytopathological Classics.

Representatives of the Society, new committees, and changes in committee personnel

are given on pages 262 and 263.
Sixty-three applicants were elected to membership in The American Phytopathological Society.

Reports of Officers, Representatives, and Committees. Reports of officers, representatives, and standing committees are published on pages 263-271. According to action of the Society at the Philadelphia Meeting reports of Special and Temporary Committees

are not to be published in the annual report. All committee reports submitted were considered by the Council. The reports recommended for approval by the Council, with slight changes in some cases, were accepted by the Society.

The Society approved the following recommendations made by the Council.

1. Contribution of \$50.00 to the Union of American Biological Societies and Biological Abstracts.

2. Commend members of the membership list committee for their excellent work and recommended that the next Council consider publication of a revised membership list in 1946.

3. Appointment of a Standing Committee called "Placement Committee" to continue the work of the Clearing Agency formerly handled by the Secretary.

4. Acceptance of a resolution relative to the importance and need of continuation of the work of the Emergency Plant Disease Prevention Program submitted to the Council

by the War Committee.

5. That the incoming President appoint a temporary committee "to study and recommend ways and means of providing for publication by the Society of useful articles not otherwise provided for with the suggestion that this committee consider underwriting acceptable publications to be sold at approximate cost to reimburse and gradually increase the Society's backlog."

6. Action of the Council taken early in 1944 in accepting the resignation of H. B. Humphrey, editor-in-chief of PHYTOPATHOLOGY, and the appointment of Helen Hart

to finish the unexpired term as editor-in-chief.

7. Appointment of E. J. Anderson, L. M. Black, F. L. Howard, and C. M. Tucker as associate editors of PHYTOPATHOLOGY for a three-year term. Appointment of L. R. Tehon as associate editor of PHYTOPATHOLOGY for 1945 and 1946; appointment of M. W. Gardner as associate editor of PHYTOPATHOLOGY for 1945. In arranging for nonconcurrent terms for the editors of PHYTOPATHOLOGY the resignation of A. J. Riker, whose term would ordinarily expire at the end of 1946, was accepted and he was reappointed for a three-year term beginning 1945.

8. Standing Committe on "Recognition of Merit" be discontinued.

9. Accept and place in effect the following recommendations presented by the Special Committee on "Publication Problems":

Place in effect as soon as possible with the present printer, Science Press, economies regarding (1) paper, (2) vertical lines in tables where this saves money, and (3) publishing in units of 16 pages in accord with its quotation of November 10. The increased cost of printing notwithstanding these quotations will provide approximately the same number of pages in 1945 as in 1941 for the same outlay.

Authorize the business manager and editor-in-chief to make the change at the end

of a volume to a double column format, in accord with the answered questionnaires, when an over-all saving of more than 10 per cent can thus be accomplished. It takes a year

or two for authors, editors, and printers to make such a change smoothly.

Ask the Society to vote the editorial staff a mandate "to sharpen editorial pencils," to employ at least two qualified and anonymous referees on each manuscript, and to improve the quality of manuscripts through suggestion and encouragement, as far as possible, and through rejection when necessary.

Authorize the preparation and publication of "Suggestions to Writers."

Charge the authors for excessive changes from galley copy at the discretion of the editor-in-chief.

Print and distribute annually, or set up as a standing page and print on otherwise blank pages, the suitable form with which a member could add a codicil and remember the Society in his will. This might provide a substantial income.

Create a class of sustaining contributors at \$100.00 per year open to industrial companies and publish the list with each number. The Society of American Bacteriologists has 60 sustaining members. The list is published with each number. A standing committee might be charged with the duty of keeping the list alive. (J. J. Christensen was selected by the Society to handle this in cooperation with the Committee on "Donations and Legacies.")

Arrange for the collection of dues in the fall and for delinquent members to have their journal stopped after the January number. This will preserve the supply of most back numbers in whole volume units, which are more valuable than units from May to May.

10. Recommended after a study of its report that the committee on Society Organiza-tion be made a Standing Committee to continue a study of the revision of the Constitution and to make specific recommendations to the Council for presentation to the Society according to the Constitution.

11. Recommended that no summer meeting of the Society be held, and leave the matter of time and place of the next annual meeting to the decision of the Council.

ARTHUR HENRY REGINALD BULLER

August 19, 1874 — July 3, 1944

Professor A. H. Reginald Buller was graduated from the University of London in 1896 with the degree of Bachelor of Science and received the degree of Doctor of Philosophy in 1899 from the University of Leipzig, Germany. The honorary degree of Doctor of Laws was bestowed upon him by the University of Manitoba in 1924, by the University of Saskatchewan in 1928, and by the University of Calcutta in 1938. Pennsylvania University granted him the degree of Doctor of Science in 1933.

From 1901 to 1904 Dr. Buller was lecturer in botany at the University of Birmingham; in 1904 he went to the University of Manitoba at Winnipeg as professor of botany, where he remained until his voluntary retirement as professor emeritus in 1936. He also held a number of special lectureships in the United States: he was Norman Wait Harris Foundation lecturer at Northwestern University in 1927, summer lecturer at Louisiana University in 1941, Hitchcock professor at California in 1942, and in the same year Schiff Foundation lecturer at Cornell.

Dr. Buller was a member of the American Phytopathological Society. His contributions to the field of mycology are so extensive, important, and well known as scarcely to require comment. Probably most notable among his contributions are those on spore formation, spore liberation, and sexuality.

ERNEST ADAM DOPP

September 12, 1896 — September 24, 1944

Ernest Adam Dopp was graduated from the University of Wisconsin in 1922 with the degree of Bachelor of Arts, and received the degree of Master of Arts in 1924 from the same institution.

Mr. Dopp was Assistant in Botany, University of Wisconsin, 1922–23; Instructor in Botany, University of Minnesota, 1924–1927, and Instructor, La Crosse (Wisc.) Normal, 1927. From 1928, Mr. Dopp served in the Bureau of Plant Industry, U. S. Department of Agriculture, as Junior Pathologist, 1928–1929, and Assistant Pathologist, 1929 to the time of his death.

Mr. Dopp was of a quiet, pleasant disposition, very modest but always most helpful to his colleagues, and thus he was a most valuable member in the various cooperative researches in which he had a part.

EDWARD FRANKLIN GAINES

January 12, 1886 — August 17, 1944

Dr. Edward Franklin Gaines was graduated from the State Normal School, Cheney, Washington, in 1907. Following a limited experience as a public school teacher, he entered the State College of Washington, where, in 1911, he received his B.S. degree. The same institution granted him the degree of Master of Science in 1913. From Harvard University he earned the Sc.D. degree in 1921.

Dr. Gaines joined the staff of the State College of Washington in 1911, under appointment as Instructor in Agronomy and as Assistant Cerealist in the Experiment Station. In 1917 he was promoted to the rank of Cerealist, and in 1930 was appointed Professor of Genetics.

Early in his experience in agronomy and plant pathology Dr. Gaines became interested in the cereal smuts and especially in the nature and control of bunt of wheat, a smut that had long exacted a heavy toll from the farmers of Eastern Washington and adjacent States. To him chiefly is due the credit for production and improvement of such bunt-resistant varieties of wheat as Ridit, Albit, and Hymar. The variety Hymar is now the most important winter wheat grown in Eastern Washington.

To his intimate friends Dr. Gaines was more than an outstanding teacher and investigator. He believed in mankind, and devoted such time as he could to the social and cultural betterment of young people. He radiated integrity, loyalty, and a rare steadfastness of purpose. He will long be remembered for his scientific attainments but longer still for his influence for good and as a man among men.

WILLIAM TITUS HORNE

November 8, 1876 — April 12, 1944

William Titus Horne was graduated from the University of Nebraska in 1898 with the degree of Bachelor of Science and was a Fellow in Botany at Columbia University, 1903–04.

Professor Horne was Instructor in Botany at Nebraska Wesleyan University, 1898–1900, and also at the University of Nebraska, 1899–1900. He was Assistant Plant Pathologist, 1904–1907, and Chief Plant Pathologist, 1907–1909, Estacion Central Agronomica, Santiago de las Vegas, Cuba; Assistant Professor of Plant Pathology, University of California, 1909–1914; Associate Professor, University of California, and Associate Plant Pathologist, California Agricultural Experiment Station, 1914–1939; and Professor of Plant Pathology and Plant Pathologist in the same institutions, respectively, from 1939 to the time of his death.

Professor Horne was a man of unusually pleasing personality and a most capable teacher and wise counselor in research. He made notable contributions in his own researches on difficult problems. The memory of his congenial companionship will linger in the hearts of his many friends.

PETER HENRY ROLFS April 17, 1865 — February 23, 1944

Peter Henry Rolfs was graduated from Iowa State College with the degree of Bachelor of Science in 1889, and received the degree of Master of Science in 1891. The degree of Doctor of Science was conferred on him by the University of Florida in 1920.

He was Professor of Natural Science at Florida Agricultural College from 1891 to 1899, Botanist and Bacteriologist at Clemson College 1899 to 1901, Plant Pathologist in the U. S. Department of Agriculture 1901 to 1906, Director of the Agricultural Experiment Station of the University of Florida from 1906 to 1921. He developed and was Director of the Escola Superior de Agricultura Vicosa, Brazil, 1921 to 1933. He was Consultor de Agricultura in Minas Geraes, Brazil, from 1928 to 1933.

Professor Rolfs was a charter member of the American Phytopathological Society. He was a pioneer in mycology and plant pathology, an interest in which he retained throughout his career, along with his wide horticultural activities. He contributed much to his associates in inspiration and stimulation of research, and to agriculturists in general in his dissemination of practical knowledge.

DEANE BRET SWINGLE

June 6, 1879 - January 18, 1945

Deane Bret Swingle was graduated from Kansas State Agricultural College in 1900 with the degree of Bachelor of Science and received the degree of Master of Science in 1901 from the University of Wisconsin and the degree of Doctor of Philosophy in 1931 from the same institution.

From 1901 to 1906 Dr. Swingle was employed in the United States Department of Agriculture, assisting Dr. Erwin F. Smith, and from 1906 to the time of his death he was connected with the Montana State College and Montana Agricultural Experiment Station. He organized the work in botany and bacteriology and in 1911, when this was recognized as a department, Dr. Swingle was made its head, in which position he remained until the time of his death. In the earlier years he also served as Acting President of the college, and in the later years, until his death, he was the Vice-president.

Dr. Swingle was charter member of the American Phytopathological Society, and was a member of a number of other scientific and social organizations. He made notable contributions in the fields of botany, bacteriology, and plant pathology; and was always a valued, sympathetic counselor to students and faculty members. Thus he erected his own memorial in the hearts of his friends.

HERBERT HICE WHETZEL

September 5, 1877 — November 30, 1944

Herbert Hice Whetzel was graduated from Wabash College at Crawfordsville, Indiana, in 1902, and pursued graduate work at Cornell University from 1902 to 1906. Wabash College gave him a Masters degree in 1906 and the honorary Sc.D. degree was destowed on him by the University of Puerto Rico in 1926 and by Wabash College in 1931.

In 1906, Professor Whetzel was appointed an assistant professor and head of a new department of botany in the New York State College of Agriculture, the name of which was changed to department of plant pathology the following year. He was appointed professor of plant pathology in 1909 but resigned his position as head of the department in 1922 in order to devote his full time to teaching and research at which he continued the remainder of his life.

Professor Whetzel was a charter member of the American Phytopathological Society, its president in 1915, and chairman of its War Emergency Board in 1918. He promoted the organization of the Mycological Society of America and was its president in 1939. He was also a member of the Botanical Society of America, of the British Mycological Society, and of several honorary societies.

Professor Whetzel possessed great energy, persistence, and enthusiasm. He was a unique character with extraordinary talents. An excellent teacher, a productive investigator, a forceful speaker, his influence was felt by students, colleagues, and friends alike and his death will be mourned by all.



HOWARD EVERETT PARSON

1897-1943

H. L. CRANE

Howard Everett Parson was born at Smith's Creek, Michigan, on May 13, 1897. He was the son of a farmer and grew up on the farm where he learned about fruits and the diseases attacking them. He attended the Beech Grade School, Wales Township, St. Clair County, Michigan. After completing his Port Huron High School training, he spent a year at Michigan State Normal School at Ypsilanti, Michigan, his schooling there being interrupted to enter the United States Army in World War I, at Ann Arbor, Michigan. He served in a Medical Company of a Casualty Detachment in England and France for approximately one year, being honorably discharged at Camp Custer, Michigan, March 1, 1919.

Upon his return to civilian life, Mr. Parson entered Michigan State Agricultural College and received his Bachelor of Science Degree in 1923. After receiving his Bachelor's Degree from Michigan State College, he was employed as Field Assistant with the Bureau of Plant Industry during the summer months of 1922 and 1924 and served in the position as Agent in the same Bureau, a portion of the year 1927. During school year, 1924–1925, he taught agriculture in Smith-Hughes School at Mesick, Michigan. This experience led him to decide to obtain additional training so that he might go into research work. In 1925 he enrolled in the Graduate School at the University of Minnesota and in 1928 obtained the Master of Science Degree from that University. His graduate work dealt with physiologic specialization in *Puccinia coronata avenae*. Part of this time he was Instructor in the School of Agriculture and Research Assistant in the Minnesota Agricultural Experiment Station. In 1927 he worked on the epidemiology of cereal rusts for the United States Department of Agriculture.

He was appointed to the position of Junior Pathologist in the Bureau of Plant Industry for work on pecan diseases, with headquarters at Thomasville, Georgia, on January 2, 1929. In this capacity, he did very important work in Georgia and Alabama, later being transferred to Shreveport, Louisiana, where he continued his investigations, working primarily on pecan leaf-spot diseases and their control, as well as on a virus disease known as bunch disease which affected the pecan and water hickory trees of that area and surrounding areas in Texas, Arkansas, and Mississippi.

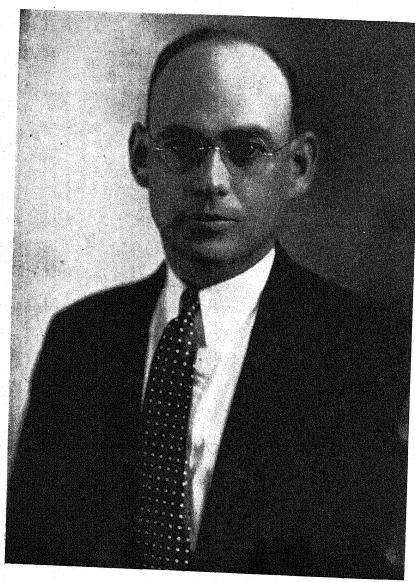
He married Adelaide Blanche Bunnell on October 6, 1930, and they have one daughter.

Mr. Parson died November 18, 1943, in Shreveport, Louisiana.

He was author or co-author of the following papers:

Physiologic specialization in Puccinia coronata evenae. Phytopath. 17: 783-790. 1927. (With J. G. Leach and H. W. Johnson.) The use of acidulated mercuric chloride in disinfecting potato tubers for the Catrol of Rhizoctonia. Phytopath. 19: 713-724. 1929. (With G. F. Moznette, C. B. Nickels, W. C. Pierce, T. L. Bissell, J. B. Demaree, J. R. Cole, and John R. Large.) Insects and diseases of the pecan and their control. U. S. Dept. Agr., Farmers' Bull. 1829. 1940.

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HOWARD EVERETT PARSON 1897-1943

ON POD SPOTS IN PEPPERS

H. BREMER

(Accepted for publication September 1, 1944)

In November, 1937, the majority of the pods in a field of peppers inspected at Bornova, near Izmir (Smyrn), Turkey, were injured by rotted spots with a layer of shining black mold on them. Microscopic examination proved the fungus to be a species of Alternaria. Pure cultures were produced on agar, one of which was identified by the Centralbureau voor Schimmelcultures at Baarn (Holland) as Alternaria longipes (Ell. et Ev.) Tisd. et Wadk.

This species of Alternaria is known to be a parasite of tobacco. Tobacco and pepper leaves were inoculated with an isolate from pepper, with negative results. Pepper fruits and stems became infected, if they had been wounded before inoculation. Thus, the fungus is a wound parasite, but it cannot be the primary cause of the spots.

In 1938, spots of the same shape but without the mold layer (Fig. 1) were frequent on peppers in the same region. They appeared in July and August, in the hottest season, on the sides or the tops of the full-grown semi-mature or mature pods.

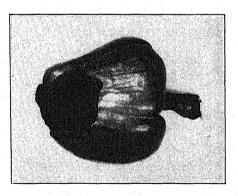


Fig. 1. Pod spot of pepper.

At first, lesions are bright-colored, somewhat sunken. In dry weather the dead skin of the spots becomes parchment-like, hard and dry; if damp weather prevails, the mold layer mentioned may develop on it. The spots often become very large, sometimes extending over almost the whole pod making it in every case unmarketable.

Pod spot is not unknown. In Florida, similar symptoms characterize "sunscald" or "blossom-end rot," in Hungary "Dorrfleckenkrankheit (dry spot disease)." In Florida the damage in some years reaches 50 per cent.

Weber, G. F. Diseases of peppers in Florida. Fla. Agr. Exp. Sta. Bul. 244. 1932.
 Szirmai, J. Die "Dorrfleckenkrankheit" (Hitzeschaden) des Paprikas. Phytopath. Zeitschrift 11: 1-13. 1938.

In Hungary 4 to 12 per cent is said to be the average loss. According to G. F. Weber, the Florida author, the possible causes are either the destroying effects of direct sun rays (sunscald) or insufficient or fluctuating water supply (blossom-end rot). This author says that the two diseases are likely to be confounded with each other but can be distinguished by the position of the spots. In sunscald the spots are on the sides of pods, while blossom-end rot appears on the top (blossom end) of the fruits. Szirmai, who studied the disease in Hungary, believes that the spots are not caused by radiation but by heat. He was able to reproduce similar spots by hot air of 50–52° C., provided that the fruits were moistened with water. Now these conditions seem rather abnormal; in direct sunlight the temperature sometimes may be 50–52° C., but moisture is not likely to be present, at least in arid regions. The results obtained by Szirmai are not to be doubted, but we cannot believe that the pod spots always are brought about in the same manner in nature.

Pod spot occurs in many pepper fields in Turkey, and sometimes up to 30 per cent and more of the fruits are made useless by the spots.

Except for secondary contaminations, fungi or bacteria could not be found in the diseased fruit tissue nor isolated from it. Therefore, the disease should be considered a physiological one, caused by adverse conditions.

The distinctions between sunscald and blossom-end rot, as given by Weber, did not always hold. In most cases the spots were lateral, often extending to the top of the pod; sometimes they were limited to the top. All types occurred in one field and close to each other, thus indicating the same origin. The symptoms of pod spot generally resemble somewhat those of blossom-end rot of tomatoes. In closely related plants, such as the solanaceous species, pepper and tomato, every local breakdown of the fruit-wall tissue may result in similar symptoms. In a few cases spots of the same appearance have been found on the tops of fruits of egg plant (Solanum melongena L.), another related species. On the other hand, it is possible that the same cause may result in different localization of spots in pepper and tomato, since their fruits are constructed differently in spite of the relationship of the two species. Pod spots in the native variety "Domates biberi" (tomato pepper), the fruits of which resemble somewhat tomatoes in their short and compact shape, were particularly similar to spots of blossom-end rot in tomatoes.

Thus, we believe that it is not possible to decide, by the external appearance of the diseased fruits, whether sunscald (heat damage) is present or blossom-end rot (the result of disturbed water balance).

No doubt the sun plays an important rôle in the origin of the damage. The injury appears at its highest degree in midsummer. In the hot days of August, 1940, many of the fruits were damaged in the experimental field of the Central Institute of Plant Protection at Ankara. All the pods that developed later remained free from disease. We tried, in the hottest days, to reproduce the disease experimentally by moistening fruits in full sunlight at noon, according to the work of Szirmai, but the results were negative.

Localization of the spots towards the sun, however, strengthens the assumption of an effect of sunrays. In most cases, as has already been observed by Szirmai, spots are on the south side of the pod. But of 28 spotted fruits in a small experimental plot (Ankara, 1940), for instance, 3 were spotted on the east side, 19 on the south side, 6 on the west, and none on the north side. These observations seem to indicate "sunscald." However, the effect of the sun cannot be such a simple one. Weber, too, supposes that other factors must join the radiation of the sun in order to produce the disease: "Sunscald is also common on varieties that show poor, little or no growth." Furthermore, "some possible contributing factors in respect to these areas are... sections protected by woods and hillsides" and "lighter, more thirsty soils."

WATER BALANCE IN PEPPER

The similarity of conditions accompanying sunscald and blossom-end rot lead us to question the effect of disturbed water balance. In 1940, at the time that pepper-pod spot was severe in the experimental field at Ankara, a heavy attack of blossom-end rot occurred simultaneously in a neighboring field of tomatoes. This raises the question as to whether there are signs of a disturbed water balance in the diseased pepper plants. To approach the solution of this question, the velocity of wilting in leaves of pepper was measured. Usually three leaves of similar area and level of insertion were cut from a pepper plant with many spotted pods and three corresponding leaves from a neighboring plant with healthy pods. To have a starting point as uniform as possible, the leaves were put in a moist chamber for half an hour, then weighed, laid out in the laboratory for drying and weighed again after definite periods. In 1938, plants were taken at random from the same variety each time, from the experimental field of Bornova. In 1939, the plants taken for the experiments were all descendants of a single plant in each variety. These experiments have been repeated nine times, and have always given a uniform result: leaves of plants with many spotted pods wilted and lost weight more slowly than leaves of plants with healthy pods. For instance, in the five experiments with descendants of single plants, the percentage losses in weight were as in table 1.

For technical reasons, it was not possible to make the readings at the same

TABLE 1.—Loss of fresh weight in wilting leaves of pepper

	Plants with h	ealthy pods	Plants with spotted pods				
	Duration of wilting	Weight loss	Duration of wilting	Weight loss			
1	Min.	Per cent	Min.	Per cent			
	81.5	15.0	80.5	2.8			
	80.5	9.0	81.0	5.8			
	29.0	1.7	31.0	1.3			
	62.0	16.1	61.5	13.3			
	79.25	21.9	78.0	16.3			

time for comparable plants in each experiment. The rate of wilting was uniformly slower in leaves from the diseased plants than in leaves from healthy plants (Fig. 2, A). In this experiment both plants from which leaves had been taken stood side by side in a plot of the experimental field. The healthy plant had 6 healthy pods, no spotted ones; the diseased plant had 4 healthy and 12 spotted pods, the immature ones not being taken into account. Loss of weight in leaves is plotted against duration of wilting in minutes. If loss of water is plotted against area of the leaves, almost the same curve results (Fig. 2, B), thus indicating that no differences in thickness of the leaves are interfering. Therefore it is to be concluded that the differences in the loss of water involved physiological differences in the plants.

If one of two genetically and morphologically homogeneous plants is more resistant against wilting than the other, we must conclude that it is or

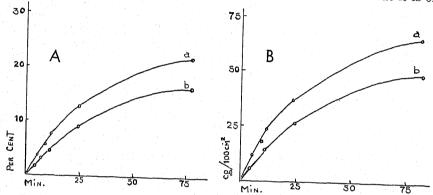


Fig. 2. A. Percentage loss of fresh weight in wilting leaves from (a) healthy pepper plants and (b) pepper plants with pod spot. B. Loss of weight per unit area of leaf.

has been influenced for some time by a factor changing its physiological state in this direction. It seems likely that the factor involved consists of one or several preceding wilting periods endured by the plant. Plants become drought-resistant by repeated wilting. Thus we surmise that pod-spotted pepper plants have been influenced previously by one or several periods of insufficient water. The same cause is assumed generally to be responsible for blossom-end rot of tomatoes. We may repeat that pod-spotting of peppers occurred, in 1940, in the Experimental Field of Ankara, at just the same time as blossom-end rot in an immediately adjacent tomato plot. Both plots had been supplied equally with water: they had been irrigated copiously at intervals of several days and thus had been exposed, in the hot and dry summer climate of Ankara, to great fluctuations of humidity and drought. Another plot of tomatoes, which has received no irrigation at all, produced at the same time a few small but thoroughly healthy fruits. From this, it becomes evident that insufficient water supply in itself does not produce the trouble. It makes the plants hardy, if they survive. Such a condition is not to be taken into account, however, in practical cultivation. On the other

hand, in the small gardens of vegetable growers at Izmir, where irrigation is practiced very diligently and regularly, and therefore periods of drought hardly occur, we almost never found pod-spotting of peppers.

We have tried to produce experimentally pod spots in potted pepper plants by irregular watering. This experiment gave no conclusive results, our equipment being poor for such purposes, and there may have been relatively great fluctuations of water supply in the checks also. In any case, pod-spotting occurred in all pots. It was noted, however, that in the potted plants in which fluctuations of water supply supposedly were greater than in the field, spotting was heavy by the end of June, while in the adjacent field the disease did not appear before the middle of July.

In a small limited area of one field, up to 37 per cent of the pods were spotted. In this case, the subsoil contained refuse of brick and stones from an adjacent building. A disturbed structure of the subsoil may result in fluctuations of the water supply.

A striking feature of the disease is the occurrence of plants with healthy and plants with heavily spotted pods close to each other in one field. For this there is no explanation, for we know practically nothing of the physiological differences of individual plants. Perhaps small differences in the functioning of the roots may play a rôle. One plant was found with all its pods spotted. Its main root had been injured, either mechanically or by an insect.

Although the data we have been able to collect are not wholly conclusive because of limitations of experimental work, we conclude tentatively that in the pod-spotting of peppers both sunscald and blossom-end rot are identical troubles. The damage to peppers is believed to occur during strong radiation of the sun, but only in plants which are or have been influenced by wide fluctuations of water supply.

From the standpoint of practical control of the disease certain facts are obvious. Against sunrays there is no protection. Shading would impair the fruiting and ripening of peppers. But it is possible to avoid fluctuations of water supply. It is necessary to provide moderate but frequent irrigations.

CENTRAL INSTITUTE OF PLANT PROTECTION, ANKARA, TURKEY.

THE PREVALENCE OF THE WHEAT NEMATODE IN CHINA AND ITS CONTROL¹

VONG-MAY CHU

(Accepted for publication September 10, 1944)

The nematode disease of wheat, caused by Anguina tritici (Steinbuch) Filipjev (= Anguillulina tritici (Steinbuch) Gervais and v. Beneden) (= Tylenchus tritici (Steinbuch) Bastian) was first found in China in the vicinity of Nanking in 1915. The actual damage caused by this disease was recognized by T. H. Shen and co-workers (15), who reported that the nematode disease of wheat is found in China from north of the Yellow River to south of the Yangtze River. The damage amounts to about 30 per cent at Hsuchow and about 10 per cent at Nansuchow.

The problems concerning distribution and control of the nematode disease have been investigated by the writer since 1933. This paper reports the results obtained up to 1942.

GEOGRAPHIC DISTRIBUTION

The distribution of this disease in China was determined by examining seed samples collected from different districts. It was considered that the results obtained by this method were reliable, since more than 88 per cent of the records obtained from sample examination corresponded with actual counts of diseased heads in several districts. Samples were collected in 1313 counties in 21 provinces throughout the territory of the Republic of China except in Tibet, Mongolia, and Manchuria. A total of 5335 samples of wheat seed were collected and examined. In addition, wheat fields in 165 counties in 20 provinces also were examined. The data show that the wheat nematode occurs in practically every wheat-growing region in China, although its prevalence varies somewhat in different provinces.

ECONOMIC IMPORTANCE

In the provinces of the main wheat belt, more than 13 per cent of the seed samples were contaminated with nematode galls, with percentage of contamination ranging from 0.01 to 5 and mostly from 0.1 to 1.0; and more than 29 per cent of the wheat fields examined were infested, with percentage of infestation ranging from a trace to 50 and mostly from 5 to 10. It was not uncommon for a single mill to discard daily 100 bushels of galled wheat out of 30,000 bushels.

The quantity of nematode galls in the seed, or the number of infected heads in the field is not an accurate index of the damage caused by this disease. Leukel (11) reported that many infected plants are killed in the seedling stage, only a few galls are developed from infected heads, and many

¹ Phytopathology extends the courtesy of its journal pages to scientists in other countries who are persevering in research under difficult wartime conditions and are temporarily deprived of the opportunity for membership in the American Phytopathological Society.

of the galls in the seed are blown out during the threshing process. During the present studies the following facts were observed: (1) When artificially inoculated seed is planted, not more than 54 per cent of the infected seedlings are able to survive until heading. (2) About 80 per cent of the badly infected heads die before maturity and produce few galls or none at all. (3) Not more than 30–90 per cent of the original quantity of galls are found in threshed grain, the remainder having been removed during threshing and refining. (4) The infected heads cannot always be diagnosed correctly. Most of the partially or lightly infected heads, and even some of the severely infected heads can easily be overlooked. In examining 5851 diseased heads of 34 wheat varieties, by dissecting all the flowers in every spikelet, only 22 per cent of the partially diseased heads and 95 per cent of the severely infected ones were diagnosed correctly.

On account of these facts, the actual reduction in yield is far in excess of that indicated by the quantity of nematode galls found in the wheat seed and in diseased heads. Experiments have demonstrated that the actual reduction in yield will amount to 5, 30, 54, and 69 per cent when the weight of the galls in the wheat seed is 0.01–0.09, 2–2.9, 6–6.9, and 8–8.9 per cent of the total weight of the mixture. According to "Crop Reports" published by the Division of Rural Economics, National Agricultural Research Bureau (1), the annual production of wheat in China is about 850,000,000 bushels. The reduction in yield caused by this disease is estimated to be about 11,-200,000 bushels.

VARIETAL RESISTANCE

Leukel was the first investigator to test the different varieties of wheat for resistance against the wheat nematode. He (11) failed to find any significant difference in the amount of infection among the different wheat varieties tested, but later (12) found that Kanred was more resistant than a number of other varieties. Leukel's observations were confirmed by S. C. Teng (see 15) who, during 1930–1931, inoculated Kanred seed and also the seed of many Chinese varieties of wheat. None of the Chinese varieties had less than 50 per cent head infection, while only 12 per cent of the Kanred variety was infected.

Experimental studies to determine varietal differences in resistance to nematode were started by the writer in the fall of 1936. At that time, in cooperation with C. Y. Chow of the National Central University, seeds of 72 Chinese wheat varieties were inoculated with galls and planted in the field. From the fall of 1937 up to 1942, the 1677 pure strains obtained by head selection in China, were added to the list, and these experiments were carried on in Chansha, Hunan, and in Kweiyang, Kweichow. Only 5 strains, selected from the tested samples, had little or no infection during the six years' experiments. Those were P.C. 876, P.C. 690, P.C. 633, P.C. 171, and Hsuchow Resistant No. 2. The maximum infection in these varieties did not exceed 0.56 per cent in weight of galls in the threshed grain, though some injury was apparent during the seedling stage. Yields of these strains have

been compared with those of the most common varieties grown in 1940–1942 in Kweichow province. P.C. 171 and Hsuchow Resistant No. 2 are not well adapted to the climatic conditions of the southwestern sections of China where their yields are rather low. The yields of P.C. 633, P.C. 876, and P.C. 690 are as good as, or even better than, the yields of commercial varieties. The main disadvantage is that they mature too late. It is desirable to have early maturing wheat varieties, in order that the wheat fields can be planted to rice on time.

PREVENTIVE MEASURES BY CULTURAL PRACTICES

Crop rotation to reduce the amount of nematode infection has been recommended (1, 7, 11). According to Leukel (11) this is valuable in North America. Fields in the infested areas that previously had grown crops other than wheat for one or two years had less infection than fields that had been planted in wheat continuously. However, the writer doubts that soil infestation is a serious problem in China, where wheat is often followed by rice. Continuous flooding of the rice fields is needed from transplanting to the ripening of the crop. In 4 years' experiments the percentage of nematode infection was highest (3.75 per cent) in the wheat crop grown from clean seed in clean soil, that in the crop grown from clean seed sown in infested soil was less (0.20 per cent), and that in the crop grown from clean seed on soil previously planted to wheat infested with nematode but with an intervening paddy crop was lowest (0.04 per cent). These results indicate that soil infestation is not important, especially when wheat is followed by rice.

CONTROL MEASURES BY SEED TREATMENT

A control program for this disease must be based on the elimination of the nematode galls that are mixed with the seed. Seed treatments that will eliminate the galls and are simple to apply appear to afford the most effective method of control. Various control measures including fanning, screening, hot water dip, chemical treatment, sedimentation by salt brine, flotation in water, and separation by means of an indented cylinder or "trieur" have been suggested by workers in different countries. Unfortunately none of these methods is entirely effective.

Fanning has been recommended by Cobb (see 7) but the effectiveness of this method has not been demonstrated. According to Coleman and Regan (7) 40 to 45 per cent of the galls remain in the wheat even if the fan revolves 850 times per minute. The writer confirmed these results and found that fanning, by the method most commonly used by farmers in China to remove impurities from the grain, resulted in complete removal of the galls from only 6 of 357 samples. The difficulty of separating the galls from the grain by this method is due to the comparatively slight difference in relative weight between the galls and the grain kernels.

Screening is the simplest way for the farmers to achieve gall eradication and is commonly practiced in China as well as in other countries. This treatment is based on the difference in size between the nematode gall and the sound wheat. Sound kernels of *Triticum polonicum* and of the wheat variety Hsuchow 405 were accurately measured as were the nearly globular nematode galls. The ratio of kernel length to gall length is approximately 5:1 for both wheats; that of kernel width to gall width is 1:0.88 in *T. polonicum* and 1:0.78 in Hsuchow 405; and that of kernel thickness to gall thickness is 1:0.71 in both wheats. In some cases several small galls may fuse to form a compound gall that is always wider and thicker than the wheat kernel. If contaminated wheat is screened or sieved some of the galls fail to pass through the sieve or a large quantity of small grains pass through. The writer found that screening 390 grams of wheat containing 0.92 per cent of galls, by a method generally used by farmers, reduced the total weight of grain to 342 grams, while 0.05 per cent of the galls remained in the mixture. These results confirm those of Coleman and Regan (7) and Jones and his coworkers (10).

The use of brine to remove the lighter infected grains from wheat seed was independently worked out by Müller in Germany and by Jackzewski in Russia in 1904. Müller used 30 per cent sodium chloride, while Jackzewski used 20 per cent sodium chloride. It was first applied in the control of wheat nematode in North America by Byars (5) in 1919. The considerable difference in specific gravity between the grain and the nematode gall, makes it possible to separate the two bodies. Although this method is considered very effective as a control measure, it is impractical in China on account of the scarcity and expense of salt in many sections of the country.

Coleman and Regan (7) suggested a method to remove the galls from sound wheat by floating infected wheat in water. Since the specific gravity of galls is 0.8125 it is not necessary to increase the density of water by using table salt. If the galls are kept in water for some time, they will imbibe enough water to sink. Observations made with galls collected from different regions show that the galls will float on the surface of fresh water for about five minutes. Consequently, the fresh water method will not be effective unless the operator can remove the galls right after the contaminated wheat is poured into the water. Although it is possible by this method to remove 96 per cent or even 100 per cent of the galls in the wheat, as well as to eliminate most of the seeds of many obnoxious weeds, it cannot be generally practiced in China for a number of reasons. The drying of moist grain is the most difficult problem, requiring considerable space for spreading and constant attention in order to avoid heating and sprouting of the grain. This is especially serious in southwestern China where the regular seeding time of the wheat crop occurs during the autumn rainy season. Another disadvantage of this method is the need for a considerable amount of water. This is a limiting factor in the mountainous districts where it is difficult to obtain water.

Since there is a difference in the heat resisting ability between wheat and the nematode gall, Bessey (2) recommended the hot-water treatment to remove the source of infection. Marcinowski (13) also proved that the nematode galls in a grain mixture can be destroyed by steeping the mixture for 10–12 minutes in water at 54–56° C. Byars (4, 5) and some other investigators (7, 10, 11) have advocated Johnson's modified hot-water method (9) which involves presoaking the seed in cold water for several hours, after which it is dipped in water at 54° C. for 10–15 minutes. The writer's experiments also demonstrated the necessity of presoaking. Presoaked galls are destroyed when kept in water at 50° C. for 10 minutes or at 55° C. for 5 minutes, whereas the dry galls can withstand the same temperatures and dipping periods. The difficulty in obtaining large quantities of hot water, and the same difficulties encountered with the floating method, make the hot-water treatment impractical for general use in China.

Chemical control measures are based on the difference in resistance against the toxic action of chemicals between wheat and the nematode gall. Davaine (8) recommended soaking wheat in 0.6 per cent sulphuric acid for 24 hours. The results of Pennetier (14), Byars (6), and Marcinowski (13), obtained with different disinfectants, agree with those of Somerville (16) who decided that the contaminated seed could not be thoroughly disinfected without injuring the wheat. The writer also experimented with some common disinfectants such as sulphuric acid, corrosive sublimate, copper sulphate, carbolic acid, and formaldehyde. Of these, the corrosive sublimate appeared most effective and copper sulphate the least. Experimental data indicate that all or nearly all the nematodes are killed after 3 hours in a 1 per cent solution of corrosive sublimate, after 4 hours in a 2 per cent solution of carbolic acid, or after 5 hours in a 5 per cent solution of formaldehyde: but they survive more than 2 hours in a 5 per cent solution of sulphuric acid and 5 hours in a 5 per cent copper sulphate solution. The resistance of galls against the action of certain chemicals varied according to their state of dryness. Dry galls are less resistant to chemicals than galls that have been presoaked. For example, when dipping dry galls in a 2 per cent solution of formaldehyde, 5 per cent survived 5 hours, but when presoaked galls were treated with a similar solution for the same time, 17 per cent survived. It is quite possible that presoaking diminishes the disinfectant qualities of the solutions. Braun (3) believes that presoaking causes the seed cells and cell walls to become saturated with water, so that the disinfectant cannot enter the organism in sufficient concentration, and the amount of disinfectant solution absorbed is also greatly diminished.

Machines for Seed Treatment

Recently Jones and coworkers (10) developed a method to separate mechanically the nearly globular galls from the oval wheat seed. A common weed-eradicating machine, a so-called "trieur," was constructed with a fully and hemispherically indented cylinder. According to these authors, their method was approximately 98 per cent effective. It is less expensive to operate than the brine treatment, it requires less skill than the hot-water

method, and is not so laborious as the water-flotation method. It may be considered, therefore, as the most reasonable and practical means to control the wheat nematode. Unfortunately the so-called "trieur" cannot be obtained in China and although this machine removes 96.7–98.9 per cent of the infectious bodies present in the wheat, it is probable that the 3.3–1.1 per cent of galls remaining may start a new epidemic.

During 1938 the writer in cooperation with S. T. Tsie, senior technician in the Department of Rural Engineering of the Kweichow Provincial Bureau of Agricultural Improvement, designed a wooden machine now known as the "wheat-nematode eliminator," which separates the nematode galls from the sound grain. It is easily constructed and the cost is within the means of the farmer's purchasing power.

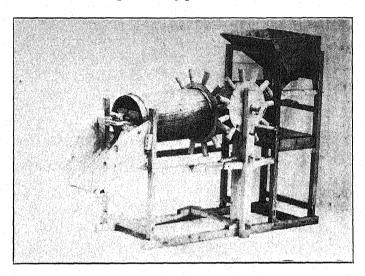


Fig. 1. Photograph of wheat-nematode eliminator.

The construction, process of manufacture, and directions for use of this machine were published in 1940 (17). Since then some improvements have been made. The improved machine is now manufactured in large quantities and is used extensively. It is made entirely of hard, light, durable, and finely grained wood, such as catalpa. It consists of a grainhopper, a grain conduct, a separating cylinder, a gall rejector, a gall receiver, a seed chute, a rotator, and a frame (Fig. 1). The separating cylinder, 80 cm. long and 25 cm. in diameter, is supported on the frame in a slightly inclined position by anterior and posterior bearings. It is turned by a rotator which consists of two ratchets, one centrally attached to the circumference of the cylinder, the other fixed on the frame at a right angle to the former. The inner-wall of the cylinder is densely and evenly indented with hemispherical concavities 4 mm. in diameter. The gall receiver is placed inside the cylinder and consists of a V-shaped tray attached to the frame by two removable bottom plates.

The V-shaped tray is placed in the cylinder so that the top of the tray will be at a height approximately two thirds of the radius above the center. The tray has a length equal to that of the cylinder and has two holes through which the galls are removed by means of a gall ejector, operated by hand, which pushes or pulls the galls to the hole in the anterior end of the tray. The galls drop through the gall rejector, a tubular chute.

The grainhopper is filled with infected wheat, and by means of the adjustor a reasonable quantity of grain falls successively through the gate into the grain conduct, and then drops to the bottom of the cylinder. The rotator turns the cylinder evenly, the grains move gradually to the anterior end of the cylinder, and the galls are separated from the grain and lifted in the cylinder concavities to the top of the tray. The galls fall through holes of the cylinder wall into the tray of the gall receiver. The ejector is then drawn to collect the galls and permit them to fall through the anterior hole in the tray into the rejector chute. The cleaned seeds move slowly to the anterior end of the cylinder and finally fall on the grain chute, which discharges them into a suitable container. A single worker may separate 34 to 45 bushels of grain within 10 hours. For the successful operation of this machine the degree of inclination of the cylinder should not be over 1.5 per cent; otherwise the separation of the galls will not be complete. Experiments have shown that only 96 per cent of the galls are removed when the cylinder is inclined in 2 per cent of its length, while the efficiency of separation may be increased to 99.7 per cent or 99.95 per cent as the inclination is decreased to 1.5 per cent or 0.5 per cent. The most satisfactory results have been obtained by regulating the speed from 60 to 95 revolutions per minute. Though the speed of revolution does not greatly affect the efficiency of gall separation, considerably more grain is wasted if the machine rotates too fast.

Several demonstrations of this equipment were given in different districts in Kweichow province during 1939–1942. In the 1941 harvest in Kweiyang and Wheisui 99.99 per cent of the plants sown from treated grain were healthy, and on the average the treatment for nematode increased the yield by 15 per cent. After this excellent showing, the writer and his coworkers conducted a demonstration campaign in districts of Kweichow province, where the wheat nematode is prevalent. A total of more than 8,000 acres were sown with mechanically cleaned seed, resulting in a yield increase of 25,000 bushels.

CONCLUSIONS

During the last 3 years various comparative experiments have been made in the methods used to control wheat nematode in China.

Fanning is practically useless since treated seed yielded 2.7 per cent galls, while in the check plot 2.4 per cent of galls were found.

Screening gives good results in controlling the nematode but may result in the loss of some of the grain.

Hot water varies considerably in the efficiency of disease control. In

some cases it reduced the percentage of infection to 0.01 per cent but in other cases as high as 4.3 per cent of galls were found.

Water flotation is efficient. This treatment reduced the disease to 0.13 per cent in contrast to 2.3 per cent in the untreated grain, and it also increased the yield by 14 per cent over that of the check plot.

Salt brine sedimentation has completely eliminated the nematode galls in treated seed lots. If there were no need to consider the expense of salt, this would be one of the most effective control measures for wheat nematode, and would compare favorably with the mechanical separation method using the special wooden gall-separating machine.

In the three years' experiments, the wheat-nematode eliminator machine has been the most effective and useful means for controlling the disease in China.

THE NATIONAL AGRICULTURAL RESEARCH BUREAU, MINISTRY OF AGRICULTURE AND FORESTRY, PEI BEH, CHUNGKING, SZECHUAN, CHINA.

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EFFECT OF LOW TEMPERATURES ON SURVIVAL OF PHYMATOTRICHUM OMNIVORUM¹

WALTER N. EZEKIEL 2

(Accepted for publication November 28, 1944)

The root-rot fungus, Phymatotrichum omnivorum (Shear) Duggar, has long been recognized among plant pathogens as one that is favored by warm weather, although it was known also that the fungus continues spreading slowly during winter along the roots of cotton plants whose tops have been killed by frost (15). Several workers determined the effect of temperature on growth of the fungus in the laboratory. Minimum, optimum, and maximum temperatures for production of sclerotia were reported (5) as 18°, 29°, and 36° C., respectively. Rogers (13) found that growth and production of sclerotia by P. omnivorum was greatest about 27° C., and still occurred at 11° C.; there was no growth at 3° C., but the fungus was not killed by exposure to this temperature for 60 days. Virulence of the fungus was retained longer in naturally-infected cotton roots stored at 1° to 2° C. than in roots stored at higher temperatures (16). Previous studies thus agree that P. omnivorum grows best at 27° to 29° C. but apparently is not injured by rather long exposure to temperatures just above freezing. There appears to have been no exploration of the effect of more intense cold on the fungus prior to the experiments reported herewith.

Many other fungi which occur in warm climates are found in cold climates also, and survive exposure to very low temperatures. Kärcher (7) listed a group of fungi (including Schizophyllum commune, Armillaria mellea, Aspergillus niger, and Penicillium glaucum) which in malt agar cultures survived exposure for 8 days at -70° C., and for 13 hours at -183° to -192° C. With the brown-rot fungus, Sclerotinia (Monilinia) fructicola, Bartram (2) found some spores and cultures to survive winter temperatures as low as -32° C., and Brooks and Cooley (3) reported slow but measurable rot of apples at 0° C.

The question has arisen on several occasions as to whether Phymatotrichum omnivorum, although favored by higher temperatures, might not also withstand exposure to severe cold. The practical question is whether this spectacularly destructive fungus would survive if introduced into areas north of the present range, or whether it might succumb to low soil temperatures during the winter.

MATERIALS AND EXPERIMENTAL CONDITIONS

Since Phymatotrichum omnivorum overwinters either as vegetative mycelium on roots or as sclerotia in the soil, both stages of the fungus were used in the experiments. The material, uniformly of isolate 24, was as follows:

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² Formerly Plant Pathologist, Texas Agricultural Experiment Station: now Senior Mycologist, Naval Ordnance Laboratory, Silver Spring, Maryland.

A. Vegetative growth in young slants, started 20 days before exposure. Cultures were on potato-dextrose agar in small tubes of 12-mm. diameter. After the period of exposure, viability was determined by making transfers from the upper half of the slants, away from the original inoculum.

B. Masses of sclerotia, produced and still in place on the walls of flask cultures (6). The flasks were placed in the various temperature compartments, and removed briefly at appropriate times for transfers to agar slants. Such transfers were made always with small portions taken from areas in the large sclerotial masses (often as large as $1 \times 3 \times 12$ cm.) that had not been disturbed previously, so each transfer was from a new and comparable portion of the sclerotial mass.

C. Cut portions of sclerotial masses, produced in flasks, placed aseptically in agar slants just before exposure to the test temperatures. The sclerotial mass was cut into pieces about $1 \times 1 \times 2$ mm., and these portions placed on the agar slants about 15 minutes before the tubes were installed in the various temperature compartments. After exposure, tubes were taken to the incubator room, where possible growth could be observed without further manipulation.

D. Cut portions of sclerotial masses, in moist soil. Portions cut as for C were placed in small cork-stoppered vials $(2 \times 5 \text{ cm.})$ of Houston black-clay-surface soil, adjusted to 34.4 per cent moisture content on an oven-dry basis. After exposure to the test temperatures, the vials were taken to the incubator room, where possible development of strands of the fungus along the walls of the vials could be readily observed, just as in the larger soil-chambers used to determine the viability of the fungus in infected roots (15).

Temperatures used in the experiments were provided as follows:

Freezing compartment,³ in which cultures were placed inside a double-corrugated-cardboard box, itself inside a metal container. Throughout the inner box, the temperature to which the fungus was exposed was rather uniformly -13° C. (8.6° F.).

Shelf in refrigerator, at 5° C. (41° F.).

Shelves in incubator room, at 28° C. (about 82° F.).

All final observations of viability of the fungus were made at 28° C., following exposure of material to lower temperatures for the periods specified. Cultures were held for several months before discarding the tubes or vials with no growth.

The periods of exposure as tabulated refer to the total time that the containers remained in the temperature compartments. Particularly at the lowest temperature there was undoubtedly a lag, perhaps of several hours before the fungus material itself cooled to the compartment temperature. Thermometers installed inside individual tubes, flasks, or such containers, and observed when material was removed, showed that the experimental temperatures were reached before the fungus was killed, but the lethal time

³ The writer was indebted to Dr. Jessie Whitacre, Chief of the Division of Rural Home Research, Texas Agricultural Experiment Station, for making space available for this work.

of exposure presumably was somewhat less than the recorded time inside the compartment.

RESULTS

Three types of material, young cultures, sclerotial masses still in place on the walls of flasks in which they were produced, and portions of sclerotial masses resting on agar slants, were exposed for periods of from 18 hours to 50 days to the 3 experimental temperatures (Table 1). Irrespective of the type of material, $Phymatotrichum\ omnivorum$ was killed by exposure to -13° C. for 2 days or longer, while some growth occurred with each type of material that had been exposed to this low temperature for only 18 hours.

TABLE 1.—Survival of Phymatotrichum omnivorum in the form of mycelial cultures, sclerotial masses, and small portions of sclerotial masses on agar slants, at the temperatures indicated (All run in duplicate, with results similar except for one pair as noted)

	eriod of xposure	20-day agar	slants		otial ma iginal fla		Scler	otia on slants	agar
-		-13° C. 5° C.	28° C.	−13° C.	5° C.	28° C.	−13° C.	5° C.	28° C
	lays	+a + + - + - + + - + + - + - + + - + - +	+ + + + + +		+ + + + + + + + + + + + + + + + + + + +	+ + + + + + +	+	+ + + + + + + + +	+ + + + + + + + +

a Growth from only 1 of the duplicate cultures.

Refrigeration at 5° C., even for a prolonged period, did not reduce growth of the fungus after transfer to 28° C. However, at 5° C. no visible growth occurred on agar slants that had been seeded with portions of sclerotial material, although the later growth after transfer to 28° C. proved that this material was viable. At 5° C. evidently growth was prohibited but the fungus was not killed.

Since in the first experiment the freezing temperature had killed the fungus in all but the shortest period of exposure, a second series was run with a single type of material, removed from the temperature compartments at more frequent intervals. Cut portions of sclerotial masses on agar slants (material C) were exposed to low temperatures for periods from 15 hours to 7 days, with the results given in table 2. Again, the refrigeration at 5° C. had no deleterious effect. Exposure to -13° C., on the contrary, reduced viability within 15 hours so that not more than 2 of 3 slants removed at any one time showed growth later, and by 39 hours had killed the remaining material.

In a third experiment, the effect of freezing under more natural conditions was considered by use of sclerotial material placed in vials of moist

TABLE 2.—Survival of sclerotia of P. omnivorum, placed on agar slants and exposed to the temperatures indicated (Run in triplicate)

Period of	Growth (+) or lack o	of growth (-) after exposure to
exposure	-13° C.	5° C.
15 hours	1.0	+
19 "	1.0	+
24 "	.1.9	+ 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1
39 '' 2 days	–	+
3 "	10 aug 1	* *
4 " " " " " " " " " " " " " " " " " " "		+ · · · · · · · · · · · · · · · · · · ·

a Growth from only 2 of the triplicate cultures.

Houston soil. This soil had not been sterilized and was fairly comparable to a favorable soil under field conditions. At -13° C. the sclerotia in the moist soil died within 19 hours (Table 3); the sclerotial masses left in the flasks survived for this period but were killed within 21 hours. At 5° C. there was uniformly no loss of viability during the 5-day-maximum exposure.

DISCUSSION

The three experiments reported have shown that *Phymatotrichum omnivorum* is highly sensitive to a freezing temperature. In none of the forms tested was the fungus viable after exposure to -13° C. for longer than 24 hours.

This sensitivity to a low temperature presumably explains the northward limitation of the range of Phymatotrichum root rot. Figure 1 shows the general area in which root rot has so far been found, from a point in southern Utah southward into Mexico (where the southern limit is yet to be estab-

TABLE 3.—Survival of P. omnivorum in the form of sclerotial masses, dry on the walls of flasks where produced, and as small portions of these masses in vials of moist soil, at the temperatures indicated (Run in duplicate)

Period of exposure		Sclerotial original			Sclerotia in moist soil in small vials			
			– 13° C.	5° C.	– 13° C.	5° C.		
21 24 26 28 43 55	()		+ + + + + + + + +		+ + + + + + + + +		

lished), and from the eastern margin of Texas westward to southern California (4, 8, 9, 10, 11, 12, 14). Superimposed on this map are two isotherms, a solid line representing an annual mean temperature of 60° F. (from Climate and Man, 1, p. 703), and a broken line delimiting the area with a lowest observed temperature of -10° F., between 1899 and 1938 (1, p. 709). These isotherms, particularly the latter, follow rather accurately the northern limit of occurrence of root rot from Arkansas west to Nevada. At the extreme western and eastern limits of this area, other conditions appear to limit spread and the temperature no longer seems the important factor.

It may be noted that the correlation of occurrence of root rot with the accumulated weather records is good also with different summations of temperature data. An average January temperature of 40° F. (1, p. 704), or an average July temperature of 80° F. (1, p. 705), or an average annual minimum temperature of 5° F. (1, p. 707), agree nearly as well with the limit

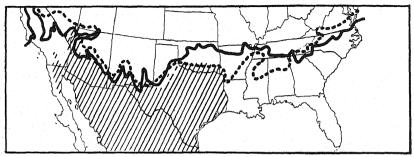


Fig. 1. Correspondence of root-rot area with recorded (1) temperature data. Shaded area, locations in which Phymatotrichum root rot has been found; solid line, annual mean temperature of 60° F.; broken line, lowest observed temperature between 1899 and 1938, -10° F.

of root-rot areas as the 60° mean temperature. An average annual snowfall of 10 inches (1, p. 727) and an average frost-free period of at least 200 days (1, p. 746) are only somewhat less in agreement.

Soil-temperature conditions suitable for continued survival of $Phymatotrichum\ omnivorum$ thus have occurred over a period of years within the climatic area delimited by the temperature indices mentioned. In other words, root rot has persisted where the temperature has not fallen below -10° F. $(-23^{\circ}$ C.), where the annual mean temperature has been 60° F. $(15.6^{\circ}$ C.) or higher, and where the frost-free period has averaged at least 200 days a year. As was noted earlier (4), the disease is increasingly prevalent, within similarly favorable soil areas and with comparable rainfall, southward from the northern limit chiefly discussed here, along with further increase in temperature. It is quite possible that the final northern limit is set by the occasional very cold periods during which the fungus may be exposed briefly to unusually low (and lethal) temperatures in the soil, in line both with the sensitivity of P. omnivorum to short periods of freezing in the laboratory, and the rather precise delimitation of the northern limit of the root-rot zone by the -10° F. line (Fig. 1) as already noted.

It seems unlikely that P. omnivorum will survive in more severe climates unless some considerable change in sensitivity of the fungus to cold should occur. The data presented suggest little reason for alarm as to the possibility of much advance of the fungus northward, either by slow growth through the soil (such subterranean spread may be at a maximum rate of about one mile in 100 years) or by more rapid introduction on the roots of diseased plants.

SUMMARY

Phymatotrichum omnivorum whether as vegetative growth on agar slants, in large sclerotial masses high on the walls of flask cultures, or as portions of sclerotial masses on agar slants or buried in moist soil, did not survive exposure in the laboratory to a temperature of -13° C. for more than 24 hours. At 5° C., growth was prevented, but there was no reduction in viability even after 50 days.

The northern limit of natural occurrence of root rot corresponds generally with several summaries of recorded temperatures, and particularly well with the line at which lowest observed air temperatures reached -10° F. (-23° C.). Coupled with the sensitivity to cold shown by the fungus in the laboratory experiments, this suggests that northward distribution of Phymatotrichum root rot has been limited by prevailing temperatures, and that the fungus is not likely to become established north of the present root-rot area.

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INFLUENCE OF TEMPERATURE ON THE DUTCH ELM DISEASE IN POTTED AMERICAN ELM

L. J. TYLER¹

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The Dutch elm disease pathogen, Ceratostomella ulmi (Schwarz) Buisman, grows little or not at all on artificial culture media at temperatures of 33° C. and higher, and at 10° C. and lower it grows poorly.2, 3, 4 Because of this behavior the influence of temperature on disease development in trees artificially inoculated with the pathogen was studied. The methods used and the information obtained are briefly summarized.

In the summers of 1940, 1941, and 1942, about 200 potted American elms (Ulmus americana L.) were used in 6 different temperature experiments. To provide for uniform response to temperature and to the disease the trees were grown from cuttings; in height they ranged from 21 to 7 feet and in age from 3 to 4 years. Except for those used in one experiment all trees had completed $\frac{2}{3}$ to $\frac{3}{4}$ of their terminal growth; the excepted trees had recently completed terminal growth before being used. In general, the vigor of all trees was good.

Six different temperature chambers were used, and, in addition, outdoor conditions were utilized. The chambers were 7 to 8 feet high by 8 to 10 feet square. The temperature series ranged from about 10° C. to about 35° C. The minimum and maximum temperatures and relative humidities recorded for each chamber in the series, and for outdoors, from that of lowest temperature to the highest were respectively: A. 9.5° to 12.0° C., 75 to 85 per cent; B. 15.0° to 16.0° C., 80 to 94 per cent; C. 18.5° to 21.0° C., 76 to 85 per cent; D. 12.5° to 20.0° C., 62 to 100 per cent (outdoors); E. 21.5° to 25.0° C., 61 to 78 per cent (basement corridor); F. 26.0° to 29.0° C., 69 to 84 per cent; G. 32.0° to 37.0° C., 66 to 79 per cent. Except for the basement corridor, blowers circulated the air in the chambers and each was lighted by a 150-watt Mazda lamp.

Inoculum consisted of distilled water suspensions of conidia⁵ from 10- to 12-day-old cultures of a virulent line of Ceratostomella ulmi grown in Petri dishes on sterile, cellulose, beer-glass coasters soaked with potato-dextrose solution. The density of the spore suspension was adjusted to about $\frac{1}{2}$ million spores per ml.

Inoculation was accomplished by means of a 10-ml. hypodermic syringe equipped with a 11/16-inch, No. LN special Becton-Dickinson & Co. needle

1 The use of facilities made available by the Boyce Thompson Institute for Plant

Research, Yonkers, New York, is gratefully acknowledged.

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5 Spores taken from coremia of the Graphium stage of the fungus.

having the delivery aperture on its side near the point. After loading the syringe with spore suspension the needle was inserted underneath the bark, on a tangent with the trunk circumference, at one point on each of two sides but at slightly different levels and about 14 inches above the soil level. As the needle was forced underneath the bark care was taken to injure some of the peripheral vessels of the wood to assure ingress. Two ml. of spore suspension were delivered at each point. The method of inoculation used was the most expeditious and effective of many earlier tried and it had no appreciably injurious effects upon the trees.

Usually the different groups of trees were placed in the temperature chambers 12 to 24 hours before inoculation. In one experiment the trees were inoculated just previous to their transfer from outdoors into the chambers. The experiments were performed during June and the first half of July in the different years.

Exposure of the trees varied from one week to one month in the different experiments; in some experiments the period was fixed and in others it was determined by the development of the disease. Data concerning wilt symptoms, temperature, and humidity were recorded each day, and at suitable intervals representative trees from the different treatments were cut and examined for discoloration in the wood. After the exposures all remaining trees in a given experiment were held outdoors until leaf fall, or until resumption of growth the following spring, after which they were cut and examined.

Air temperature exerted a marked influence on the development of the disease in the artificially inoculated, potted elms. The range of temperature generally favoring disease development extended from about 15° to 29° C. Trees exposed to temperatures that fluctuated daily between 26° and 29° C. had 100 per cent wilt in 3 to 5 days, and the extent of wood discoloration showed that invasion by the pathogen was complete or nearly so. Exposure of inoculated trees to temperatures of 32° to 37° C., or at 9.5° to 12° C. definitely retarded or even inhibited wilt; usually at least 2 weeks elapsed before wilt appeared and then it occurred only in minor amounts. The invasive activities of the pathogen, as marked by discoloration in wood, likewise were almost entirely suppressed by the high and low temperatures.

The results obtained by shifting inoculated trees from temperatures favorable to the pathogen to those unfavorable and vice versa also emphasized the influence of temperature on the development of the disease. For example, symptoms were delayed 2 to 5 days in inoculated trees that were held at about 27° C. for 4 days and then shifted to a chamber held at about 10° C. On the other hand, in two groups of inoculated trees, each held for 4 days at a temperature distinctly unfavorable for the pathogen, the symptoms were speeded by 2 to 4 days over those of inoculated checks (check trees held constantly at unfavorable temperatures) by shifting them to the more favorable temperature of 27° C. The removal of inoculated trees from temperatures unfavorable for the development of the disease, such as

10° C. and 32° to 37° C., to the outdoors after 1, 2, and 3 weeks exposure ultimately resulted in complete wilting and extensive fungous invasion if the trees were in an active state of terminal growth at the start. If the trees had completed their terminal growth before exposure to unfavorable temperatures, the disease often was definitely suppressed in some trees and never developed at all in others even after they were removed to the outdoors.

In trees that had completed terminal growth shortly before they were inoculated, the disease was completely inhibited by a 15-hour exposure at night (5 p.m. to 8 a.m.) at 32° to 37° C. alternated with a daily 9-hour, outdoor, daytime (8 a.m. to 5 p.m.) period at temperatures that ranged from 10° to 33° C. The reverse treatment, i.e., indoors at high temperature during the day and outdoors at cooler temperatures at night, permitted complete wilting of similar trees in from 14 to 26 days. Inoculated trees kept constantly outdoors as checks during the same period wilted completely within 2 weeks; inoculated trees kept constantly at 32° to 37° C. as checks did not become diseased but their foliage was severely injured by the high temperature. Foliage of trees in the groups that were alternated daily between the two temperatures was not injured by high temperature.

The results, which are not in accord with those of Buisman,⁶ show that pathogenic activities of *Ceratostomella ulmi* in potted elms may be definitely altered by temperature. Temperatures known to suppress saprogenic activity, likewise were inhibitive to pathogenic activity, while a temperature that was optimum for the organism as a saprogen also speeded its activity as a pathogen.

DEPARTMENT OF PLANT PATHOLOGY,

CORNELL UNIVERSITY,

ITHACA, NEW YORK.

⁶ Buisman, Christine. Verslag van de phytopathologische onderzoekingen over de iepenziekte, verricht in het Laboratorium, Willie Commelin Scholten, gedurende 1931. Tijdschr. Plantenz. 38: 17–36. 1932.

SOME ETIOLOGICAL ASPECTS OF MEALYBUG WILT¹

WALTER CARTER

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The writer's first paper on the etiology of mealybug wilt (1) concluded that the evidence "pointed to a nonliving toxic insect secretion as causal." In later papers (2, 3, 4), this conclusion was reaffirmed, while in 1939 a summary of the investigations was included in a review of injuries to plants caused by insect toxins (5). Many of the findings reported herein were made in connection with a study of the effect of fertilizer on susceptibility of pineapple plants to mealybug wilt.

Topics for discussion in this report include: symptom expression and the period for the development of symptoms; the recovery of plants from mealy-bug wilt and the relation of repeated mealybug infestation thereto; the susceptibility of recovered plants to a second infestation of mealybugs; the localization of wilt in experimental plots; the effect of sunlight on susceptibility; and finally, some data on infested planting material and an infestation method.

SYMPTOM EXPRESSION

Designation of developing symptoms into four classes with a fifth indicating a recovery stage has proved practicable. Stage 1 is the preliminary In stage 2, a definite color change from red to reddening of the leaves. pink occurs, as well as the reflexing of the leaf margins. In stage 3, the affected leaves have lost their turgor and have drooped, while in stage 4, the affected leaves have dried up for the greater part of their length. A relatively small proportion of cases develop to stage 4, stage 3 usually being followed by the recovery stage. After plants have recovered and the oldest affected leaves become senescent, the youngest affected leaves, having then grown out, will be wilted at the tips only, while the new center leaves are apparently growing normally. This stage has been designated by the initials TWNC. If plants wilt when showing inflorescence or green fruit, the drying up of these is accompanied by the development of the first three wilt stages in the innermost leaves or the bracts at the base of the developing fruit.

Following the course of the disease over many thousands of plants, the expression of symptoms on one or two leaves only of the plants has been noted from time to time. This limited expression may reach any one of the first three stages of wilt. Some of these cases progress to typical third stage wilt, but in others no further development of symptoms occurs. The incidence of these cases is shown in table 1, and it should be noted that they did not occur in plants having only a short period for the development of symptoms.

¹ Published with the approval of the Acting Director as Technical Paper No. 154 of the Pineapple Research Institute of Hawaii, University of Hawaii.

TABLE 1.—The incidence of wilt cases in which symptoms were limited to (A) 1 or 2 median leaves only and (B) 1 or 2 median leaves followed by complete wilt

		No. of plants					
Age of plants when infested	No. days after infestation	TTT'43	With limited wilt				
		With wilt	A	В			
Test 1 5 months	55 68 104 134 165	1,065 3,559 820 160 23	0 0 5 10 8	0 0 31 5			
$Test \; \mathscr{Z} \ 5 \; ext{months}$	43 49 58 70 78 105 125	34 353 1,070 3,637 1,064 286 92	0 0 0 0 0 0 23 4	0 0 0 0 0 75			
Test 3 9½ months	56 63 82 90 125 145 181 215 246	3 102 298 104 336 162 154 74	0 0 0 0 1 1 2 2	0 3 40 32 46 13 19 0			

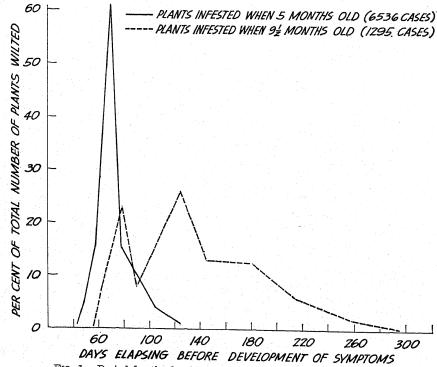


Fig. 1. Period for the development of symptoms of mealybug wilt.

PERIOD FOR DEVELOPMENT OF SYMPTOMS

The term "incubation period" cannot be properly used to describe the period between the feeding of mealybugs and the first symptoms of wilt, since there is no evidence whatsoever of a period of increase for any causal entity. Insofar as the term "latent period" carries the same connotation, the same objection holds. It has been previously indicated that a period of approximately two months is necessary between the insects' feeding and the appearance of typical wilt symptoms, but our information on this has now been considerably extended.

Tables 2 and 3 and figure 1 present the data for two extensive factorial experiments in which detailed records were kept of the development period.

TABLE 2.—Variation in the time required for the development of symptoms of mealybug wilt and distribution of the 6,536 cases according to factorial treatment. Plants infested when five months old

Treatment		No. of	plant		vilted from		25 days	
	35	43	49	58	70	78	105	125
Low Fe	0	9 25	181 172	558 512	1,799 1,838	572 492	$\frac{134}{152}$	36 56
Low NHigh N	0	11 23	$\frac{126}{227}$	$\frac{443}{627}$	$1,623 \\ 2,014$	708 356	203 83	61 31
Low KHigh K	0	$\begin{array}{c} 16 \\ 18 \end{array}$	$\begin{array}{c} 161 \\ 192 \end{array}$	$519 \\ 551$	$1,858 \\ 1,779$	539 525	142 144	36 56
No chloropicrin Chloropicrin	0	$\begin{array}{c} 9 \\ 25 \end{array}$	$\frac{100}{253}$	$\frac{260}{810}$	1,777 $1,860$	868 196	189 97	63 29
Totals for all plots combined	0	34	353	1070	3,637	1,064	286	92
Percentage plants wilted for all plots combined	0	0.52	5	16	56	16	4	1

Table 2 is from a series which was infested when the plants were five months old (7, Factorial Test 2), and the distribution of most of the cases between 58 and 77 days is clearly shown, with a mode at 70 days. It should be noted also that there was a decided tendency for the disease to appear in a shorter time in plants grown at high N levels and in chloropicrined soil. Table 3 presents a similar set of data for a test with plants which were infested at nine and a half months old (7, Factorial Test 3) and shows no clearly defined peak as with younger plants, but rather a scattering over 82 to 181 days, with considerable numbers of cases showing a still longer period. All of the late cases were those in which wilt symptoms first appeared when the fruits were green.

RECOVERY

Frequent reference has been made to this phenomenon. If a wilted plant is kept free of mealybugs, recovery from the center always occurs, although it may be, and usually is, slow if the plant originally went to the fourth stage of wilt. Typically also, the originally wilted leaves remain on

TABLE 3.—Variation in the time required for the development of symptoms of mealybug wilt and distribution of the 1,295 cases according to factorial treatment. Plants infested when 9½ months old

	No.	of pla	nts wh	ich wilt	ed fro	m 56 to	295 da	ys af	ter in	festat	ion
Treatment -	56	63	82	90	125	145	181	215	246	258	295
Low Fe	$\frac{2}{1}$	53 49	157 141	61 43	177 159	77 85	93 61	28 46	13 19	17 10	0 3
Low N High N	2 1	56 46	$\begin{array}{c} 155 \\ 143 \end{array}$	$\frac{60}{44}$	$\begin{array}{c} 176 \\ 160 \end{array}$	86 76	97 57	49 25	$\begin{array}{c} 11 \\ 21 \end{array}$	$\frac{13}{14}$	$\frac{2}{1}$
Low K	0 3	49 53	$\frac{154}{144}$	58 46	$\frac{166}{170}$	83 79	90 64	$\frac{33}{41}$	18 14	$\frac{14}{13}$	3
No chloropicrin Chloropicrin	0 3	39 63	$\frac{143}{155}$	51 53	$\begin{array}{c} 165 \\ 171 \end{array}$	79 83	84 70	36 38	$\frac{15}{17}$	$\begin{array}{c} 14 \\ 13 \end{array}$	$\frac{1}{2}$
Totals for all plots combined	3	102	298	104	336	162	154	74	32	27	3
Percentage plants wilted for all plots combined	0.23	8	23	8	26	13	12	6	3	2	0.23

the plant until senescent without loss of symptoms. In recent large-scale experiments under frequent observation, a considerable percentage of plants showed the first stage of wilt only, namely, the slight all-over reddening of the leaves, and on subsequent observation this symptom had disappeared and the plant was apparently normal. Data concerning this observation (Table 4) are from the factorial test 2 of the preceding paper (7), which can be referred to for details of the treatment. There was no apparent relationship between the number of plants wilted in any replication and the number of plants that recovered from first stage wilt. It is clear that there is no relationship between fertilizer treatment and this phenomenon, but it is equally clear that the differences between plots are very significant.

Wilt symptoms are associated with the death of roots, and recovery with

TABLE 4.—Summary of cases in which symptoms did not progress beyond first stage and recovery was rapid

Treatment	Total plants wilted	Total plants recovered from 1st stage wilt	Percentage	
N1 K2 S1 F1 2 2 2 1 2 1 1 1 1 1 2 1 1 1 1 1 2 1 2 1 2 1 2 1 2 2 1 1 2 2 2 1 1 2 2 2 1 1 2 2 1 2 2 2 1 2 2 2 1 2 2 2 1 2 2 2 1 2 2 2 1 2 2 2 1 2 2 2 1 2 2 2 1 2 2 2 1 2 2 2 1 2 1 2 1 2 1 2 1 2 1 2 2 1 1 2 1 2 1 2 1 2 1 2	426	33	7.7	
	374	35	9.4	
	433	71	16.4	
	447	20	4.5	
	361	46	12.7	
	429	8	1.9	
	420	84	20.0	
	399	58	14.5	
	445	14	3.1	
	374	23	6.1	
	444	77	17.3	
	445	33	7.4	
	384	41	10.7	
	349	45	12.9	
	374	30	8.0	
	433	63	14.5	

the production of new roots, but the disappearance of the first stage symptoms, which, incidentally, require considerable experience for their correct determination, can perhaps be explained on the basis that in such cases, only a portion of the root system had been affected, or that new roots were being produced distal of the affected zone of the plant during the period for development of symptoms. It must also be evident that if transient first stage symptoms only can appear, it is very probable that damage to the plants might occur without visible symptoms.

Recovery may be affected by nitrogen fertilization (Table 5). These data were obtained in connection with a test of the effect of nitrogen fertilization on susceptibility (7, Nitrogen Test 1) and show that plants in section 1, infested when approximately three months old, recovered and fruited in direct proportion to the amount of nitrogen supplied. Recovery in plants

TABLE 5.—Effect of nitrogen fertilization on recovery of wilted plants to fruiting stage

	Nitrogen	Section 1, infested Jan., 1937	Section 2, infested April, 1937	Section 3, infested June, 1937
Percentage of plants wilted	0 100 500 1,000	72.65 73.73 68.33 72.73	29.57 8.77 20.69 19.13	19.30 18.42 19.47 15.65
Percentage of wilted plants fruited	0 100 500 1,000	24.71 33.33 47.57 48.27	55.88 50.00 33.33 27.27	63.64 47.62 54.55 33.33

in sections 2 and 3, infested later at approximately six and eight months old respectively, shows an almost converse trend. Notes on recovery were taken up to November of the plant crop year, or four to five months after the normal harvest season.

The plants which wilted when only three or four months old had only the first flush of roots affected and new roots permitted recovery in proportion to the nitrogen supply. Plants infested and wilted later had had a much larger percentage of the available root "buds" developed and therefore damaged. In the large soft-leaved plants of the higher nitrogen series collapse was complete and "flabby," more so than in the lower N plants which were harder and higher in carbohydrate reserves. The former were much slower in recovering.

A relationship between intensity of infestation and extent of recovery has been noted recently. Two contiguous beds of pineapple plants were infested with mealybugs, one bed three times at monthly intervals and the other bed once only. In both cases 100 per cent of the plants had typical wilt symptoms. The relative recovery of these plants, however, was of quite a different order, the series infested only once showing very real recovery compared with the more heavily and frequently infested series (Fig. 2).

The most logical explanation of this difference is that the more frequently infested plants received three separate successive injuries to the roots which would materially reduce the number of root "buds" available to the plant for recovery. This would seem to be a more logical explanation than to ascribe it to mass action per se, since mass action presumably must occur at the same place and time.

Another observation pertinent to this question, however, indicates clearly that mass action is a factor. It used to be the practice when harvesting pineapples to pile the crowns that were cut from the fruits at harvest time

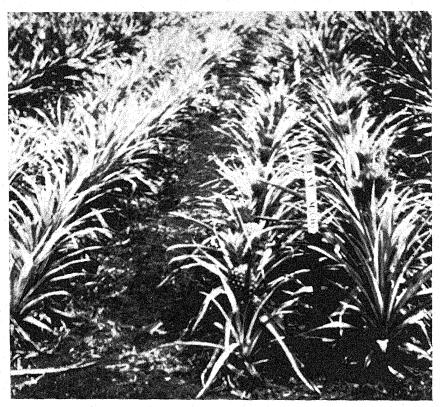


Fig. 2. Recovery from severe wilt in plants infested only once (right) compared with that in plants infested three times at monthly intervals (left).

on top of the developing ration suckers at the ends of the beds. This very frequently resulted in wilt in these suckers which became infested from the mealybugs leaving the drying crowns. As it was frequently observed, the wilt at the ends of the beds was out of all proportion to the scattered wilt incidence that may have occurred in the fields from which these crowns were collected. A recent case in point recalled this old practice and gave an opportunity for detailed observation. Figure 3 shows the end of a pineapple bed in which the ration suckers are typically wilted. This bed had had the crowns from the fruit harvested in that area piled on it at the end.

Careful examination of the plants from which these crowns came showed not a single case of wilt in any of the plants. It was clear from this that populations of mealybugs inadequate to cause wilt in the plants from which they came were sufficient to do so when concentrated on a few plants at the end of the beds. Experience with field control, however, adds a large body of indirect evidence to these specific observations. Control measures have been developed which, when properly applied, prevent the development of populations.

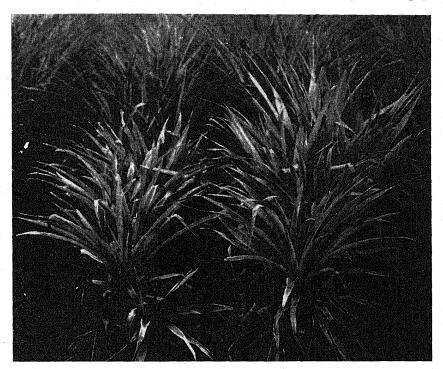


FIG. 3. First ration suckers wilted as a result of piling infested crowns from plant crop fruits at the end of the line.

lations sufficient to cause wilt but they do not eliminate all the bugs. When these controls are discontinued or inadequately applied, these small populations rapidly increase with the result that wilt develops on local areas comparable to that obtaining prior to the development of control measures.

THE SUSCEPTIBILITY OF RECOVERED PLANTS TO A SECOND GROSS INFESTATION OF MEALYBUGS

Detailed data concerning this have been presented in the preceding paper (7), because it was in connection with the second wilting, that fertilizer treatment appeared to have some significant effect. The fact that a recovered plant is just as susceptible to wilt by a second heavy infestation of mealybugs as is one which showed no symptoms following the first infestation, is a clear datum supporting the hypothesis that mealybug toxin does

not reproduce in the plants and that new tissue is toxin-free. The symptoms in the second wilting were precisely the same as in the first.

THE EFFECT OF EXPOSURE TO SUNLIGHT

It has been recognized for some time that light was an important factor in the expression of wilt symptoms. Even in a relatively open greenhouse covered with a heavy grade of reinforced glass, which no doubt radically affects light quality and intensity, conditions are so unsatisfactory for the expression of typical wilt that the use of such houses for laboratory experiments on wilt has long since been abandoned. In four factorially arranged experiments, in two of which the plants were infested when about five months old and in two when the plants were nine and one-half months old, it was evident even to the casual observer that in the plants infested when they were quite young and small, the incidence of wilt on the south side of the two-row bed was greater than on the north side. This observation is supported by the statistical analysis of the data which reveals a significant increase in the wilt incidence on the south side of the bed. Wilt incidence in plants infested at nine and one-half months old, when they had attained considerable size, did not show this significance. These data are in table 6.

TABLE 6.—Chi-squared (χ^2) test of wilt incidence on north and south sides of two-row beds

Test No	1	No. of wilted plan	9	70 1 1 1111	
rest No	North row	South row	Total	χ^2	Probability
1a	2,691 3,175	2,934 3,374	5,625 6,549	10.5 6.0	< 0.01° < 0.02°
3 _p	659 364	636 383	1,295 747	$0.4 \\ 0.5$	$ \begin{array}{l} $

a Plants infested when five months old.

c Significant.

LOCALIZATION OF WILT IN EXPERIMENTAL PLOTS

This localization has been encountered in greater or less degree in every field experiment on mealybug wilt for the last 12 years. Figure 5 is a diagram of all the plants in an experiment on fertilization in relation to susceptibility which was arranged in factorial design (7, Factorial Test 4). All the plants in these plots were infested on the same day with very heavily infested pieces of fruit rind, so that if the plants were equally susceptible at the time they were infested, the distribution of wilt should have been much more uniform. The fact that wilted plants are grouped in localized areas to the extent that this figure shows, suggests again the presence of a factor affecting susceptibility that can only be ascribed to the biological soil complex. Evidence was obtained that these local areas where wilt incidence is high are not stable, when one large field factorial experiment was duplicated exactly both with respect to treatment and location of the plots in the

b Plants infested when nine and a half months old.

area. Although localization occurred in both experiments, that in the second one did not coincide with that which occurred in the first. Differences in the physical state of the soil or of the soil itself would seem, therefore, to be ruled out. There is a very clear relationship between root collapse and wilt, and any hypothesis to account for this localization should consider the possibility that the plants in those locations were rendered more susceptible to mealybug wilt as a result of microbial activity.

There is sufficient evidence, however, to indicate that well-developed root systems of themselves are not adequate to reduce susceptibility in young plants, for it has been amply shown that chloropicrin treatment of the soil does not affect susceptibility of young plants to wilt (7) and it is evident that whatever the soil factor may be which affects susceptibility, it is not one which is affected by chloropicrin treatment of the soil. Although no formal tests have yet been made with D-D mixture in this connection (6), there have been sufficient cases of incidental wilt occurring in plots treated with D-D mixture to indicate that this material may have no more effect in reducing susceptibility in young plants than does chloropicrin.

Except for a few specific organisms, very little is known concerning the biological complex in pineapple soils, and still less about species interaction. The best evidence that the state of the soil affects susceptibility is to be found in the observation, frequently repeated, that pineapples grown in virgin land are definitely less susceptible to mealybug wilt than are plants grown in old pineapple areas. This is true whether the area in question is far removed from previous pineapple cultivation or whether it occurs as a result of relocating field margins to include small sections of contiguous virgin land. Less frequently observed has been a similar result in land treated with chloropicrin before planting. Mealybug infestations in the few such cases were not recorded in the early stages of growth of the field, since the observations were not made until the fields were in first ration stage and showing dense growth, but the presumption is that they were low.

MEALYBUG INFESTED PLANTING MATERIAL IN RELATION TO LATER SUSCEPTIBILITY OF THE GROWING PLANT

When mealybug-infested planting material is planted in a properly prepared field, most of the bugs usually disappear in a short time.

Experiments were set up to test the influence of such planting-material infestations on the susceptibility of these same plants to wilt following mealybug infestation at a later stage of their growth. The pieces of planting material from 34 mother plants were planted in clones at which time a record was made of the mealybug population on each piece. Twenty-one mother plants had either all or only part of the planting pieces infested with mealybugs at the time of planting, while 13 mother plants had no infested planting material. Shortly after growth had started, the mealybugs on the infested plants were eliminated in the usual manner and all plants were maintained free of mealybugs until experimental infestations were made.

It is clear that the previous infestation of the planting material did not affect the percentage of plants that wilted following later mealybug infestations, for of the 13 mealybug-free clones, 62 per cent of the plants wilted, while of the mealybug-infested clones, 61 per cent of the infested plants wilted and 57 per cent of the mealybug-free plants wilted.

THE FRUIT RIND INFESTATION METHOD

This technique for large-scale infestations consists essentially of selecting infested green fruits, cutting off the rind and dropping small pieces into the hearts of plants to be infested. The method was checked to determine whether mealybug colonies from individual fruits differed in toxicity. The pieces of rind from single fruits were applied consecutively to plants in the row and when wilt had developed, the diagram of the wilt incidence was

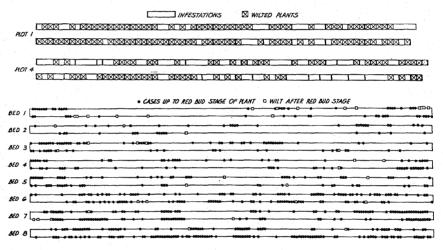


Fig. 4 (top). Diagram showing plants infested with mealybugs by the fruit rind method, using pieces from single fruits to infest consecutive plants in the row.

Fig. 5 (bottom). Diagram showing position of all the wilted plants in a field plot in which the plants had been infested with mealybugs by the fruit rind method with pieces selected at random.

superimposed on that for the single fruit infestation. There was no indication that any differences existed in the toxicity of individual fruit colonies. Figure 4 is a diagram of a typical section of the result obtained for several thousand plants. This figure should be compared with figure 5 which diagrams the total wilt incidence in a large-scale experiment in which pieces of fruit rind from hundreds of fruits were used at random. The patterns of wilt incidence are essentially the same in both figures.

SUMMARY

Observations and data on the etiology of mealybug wilt are presented. Symptom expression can conveniently be designated in 4 progressive stages and 1 recovery stage. Limited expression of symptoms may occur and 1 or 2 leaves only be affected if the period for the development of symptoms is

prolonged. The period for the development of symptoms, which varied from 43 to 295 days, is affected by the age of the plant at time of infestation.

Recovery from advanced stages of wilt occurs but that from first stage wilt can occur with complete loss of symptoms. Recovery may also be affected by N fertilization but the age of the plant at time of infestation is a factor. Frequent mealybug infestation adversely affects recovery. There is additional evidence that mass action is a factor and that sub-wilting mealybug colonies, when joined, will cause wilt.

Recovered pineapple plants are susceptible to later mealybug infestation and will wilt a second time, with typical symptoms. Reduced wilt incidence in glass houses and on the north side of two-row beds suggest that light exposure is a factor in susceptibility. Wilt in field plots is localized and diagrams showing the pattern of wilt incidence are presented. When infested planting material was planted and the mealybugs removed, no effect of those mealybug colonies on susceptibility to later infestation was demonstrable.

PINEAPPLE RESEARCH INSTITUTE, UNIVERSITY OF HAWAII, HONOLULU, HAWAII.

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THE INFLUENCE OF PLANT NUTRITION ON SUSCEPTIBILITY OF PINEAPPLE PLANTS TO MEALYBUG WILT1,2

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WALTER CARTER

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INTRODUCTION

For the past several years experiments have been conducted on the relationship between plant nutrition and susceptibility to mealybug wilt of pineapple plants. The primary object of these tests was to determine whether or not susceptibility could be reduced under field conditions by modifications of the prevailing fertilizer practice. Initially, tests were with plants grown at varying levels of nitrogen, potash, calcium and iron fertilizer, each element being considered in a separate experiment. In later experiments, combinations of nitrogen, potash, iron, and partial sterilization of the soil were studied. All experiments were in the field on a scale sufficiently large to permit statistical analysis of the data and at the same time to afford an unusual opportunity for observations on the etiology of the disease. The latter are recorded in a concurrent paper.

TESTS WITH NITROGEN

Test 1

In this test the fertilizer schedule called for four variants of the nitrogen supplied, namely, no nitrogen, 100, 500, and 1,000 lb. per acre, applied in aliquots of 100 lb. each, beginning in November, shortly after the field was planted, and continuing, for the 500- and 1,000-lb. plots, bimonthly and monthly, respectively. All the plots received superphosphate and potassium sulphate uniformly. Plots were arranged so that three periods of infestation could be used and for each period there were six replications of each treatment. On account of the fertilizer schedule, there was actually a different N variable for each infestation period, the final aliquot for all the 500- and 1,000-lb. plots being applied after the last infestation.

A standard method of mealybug infestation was used. infested pineapple plants were collected from fields showing wilt and the medium-sized mealybugs were taken from them in large numbers. From this mass of mealybugs, aliquots of 50 were put into separate vials, usually in the late afternoon, and used to infest individual plants the following morning. Infestations were sprayed out after two weeks had elapsed. Following infestation, the plants were kept under observation until they fruited the following year. There was no relationship between N level and wilt incidence. About 70 per cent of the plants wilted when infested at approxi-

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mately three months old, but there was a sharp drop in the percentage wilted in the plots in which the plants were infested at five months and seven months of age, confirming previous evidence that susceptibility decreases with age of plant.

Test 2

In this test, nitrogen levels of 0, 500, and 1,000 lb. to the acre were used with a uniform application of 300 lb. per acre each of phosphorus and potassium. There were 28 plots of 20 plants each and all the plots were planted with slips of a single clone on October 27, 1938. Fifty mealybugs per plant were applied on April 20–22, 1939, approximately six months after planting, and were allowed to feed for approximately two weeks. Final wilt readings were taken September 10, 1939. Plots were kept under observation until fruit harvest and no change in the incidence of wilt occurred. In this test the average percentages of wilted plants were 34, 32, and 19, the latter being in the 1,000-lb. N plots. This difference was significant at odds of 19 to 1.

Test 3

On September 26, 1940, following fruit harvest of plots of test 2, the first ration suckers on the mother plants that had failed to wilt when infested six months after planting, were reinfested. The sucker production in the nonitrogen plots was small, as could be expected, but every well-developed sucker was infested with mealybugs. In this infestation a different technique was used, in that a piece of the rind from heavily infested fruits was dropped into the center of each plant. About three weeks later the mealybug colonies established on the plants were sprayed out. This meant a considerably heavier infestation than the standard 50-mealybug lots used previously, but the method permits the infestation of the plants in a more natural manner without excessive handling of the insects and with the reduced labor involved makes it possible to infest on a very much larger scale. The number of mealybugs applied by the fruit rind method is always in excess of that shown by previous experiments to give high percentages of wilted plants, although there is considerable variation between individual pieces.3 The result from counts of the mealybugs on 15 pieces of fruit rind taken at random showed the number of gravid females to vary from 1 to 22, the medium-sized bugs from 24 to 102 and the crawlers from 140 to 690, with a minimum number of all classes of 165 on a single piece of fruit rind.

TEST WITH POTASH

Test 1

This test was in the same field and in the beds contiguous to those used for nitrogen test 2. All the plots were planted to the same clone, and received uniform applications of nitrogen and phosphorus. Potash was varied

 3 Carter, Walter. The toxic dose of mealy bug wilt of pineapple. Phytopath. 27: 971–981. 1937. 41 1

at 0, 300, and 600 lb. per acre. The plots were planted October 27, 1938, and each plant infested with 50 mealybugs March 29, 1939, at which time the potash applications had been completed. In August, 1939, all the plants which had failed to show symptoms were reinfested with a second dose of 50 mealybugs. Plant analyses made at that time revealed material differences between treatments, with respect to potash and nitrate N, both of which increased with increased amounts of potash applied. Wilt in the 300-lb. plots showed a significant increase (odds of 19 to 1) over that in the no-potash and 600-lb. plots.

Test 2

This experiment corresponds to test 3 of the nitrogen experiment in which the suckers growing on the previously infested plants were infested grossly by the fruit rind method. Wilt incidence was uniformly high as in nitrogen test 3, which was concurrent.

TESTS WITH CALCIUM

In these tests, 25 cc. of a 5 per cent solution of calcium chloride were applied to the axils of the base leaves of field grown plants weekly, fortnightly, and monthly. Other fertilizer applications were uniform. These applications were made from February to March 22, 1939, on plants planted late in October of the previous year. All the plants were infested with 50 mealybugs per plant on April 13, 14, and 15 but no significant differences in wilt incidence resulted.

TESTS WITH IRON

These tests were run parallel with the nitrogen, potash, and calcium experiments. The plants were fertilized uniformly but there were three variations in the iron treatment: (1) no iron spray, (2) iron spray every two weeks, and (3) iron spray twice weekly. A 4 per cent solution of ferrous sulphate was applied in a fine mist shortly after planting in October, 1938, and continued until the end of the experiment. Fifty mealybugs per plant were applied April 26, 27, and 28. Iron deficiency was becoming evident in the no-iron plots at the time infestations were made, and pronounced differences developed thereafter. The plots sprayed with iron twice a week, an excessive quantity according to plantation practices, nevertheless responded to the increased iron supply. There were, therefore, three quite clearly defined differences in greenness of the plants when the mealybugs were applied and the differences were maintained throughout the experiment, but no significant differences in wilt incidence occurred. Infestations of the young first ratoon suckers in both the calcium and iron plots were made at the same time as were those in the nitrogen and potash experiments with no significant differences resulting.

TESTS WITH NITROGEN, POTASH, IRON, AND PARTIAL STERILIZATION OF THE SOIL IN COMBINATION

These plots were arranged in a factorial manner to permit more adequate

statistical analysis of the results. Furthermore, the experiments were on such scale that from 7,000 to 9,000 plants were involved in each test. Table 1 applies to all the experiments in this series, and shows the plot arrangement.

TABLE 1.—Experiments on the effect of nitrogen, potash, iron and soil sterilization on susceptibility. Diagram showing factorial arrangement of plots²

									-					
L	w F	е	Plot	Hi,	gh F	e	Plot	Hi	gh F	e	Plot	Lo	ow Fe	3
N1	K2	S1	17	N1	K2	S1	33	N1	K2	S2	49	N1	K2	S1
		2		1	1	2	34	1	1	1	50	2	. 1	1
2	1	1	19		1	1	35	2	1	2	51	2	2	- 2
1	1	2	20	2	2	2	36	2	2	1	52	1	1	2
1	1	1	21	2	1	2	37	1	2	1	53	2	2	1
2	1	2	22	1	2	2	38	2	1	1	54	2	1	2
2	2	1	23	2	2	1	39	1	1	2	55	1	1	1
. 1	2	2	24	1	1	1	40	2	2	2	56	1	2	2
Hi	gh F	e	Plot	L	ow F	e	Plot	L	ow F	e	Plot	Hi	gh F	e
2	1	2	25	2	2	2	41	1	1	1	57	1	1	2
1	1	. 1	26	2	1	1	42	2	2	1		1	2	1
2	2	1	27	1	1	2	43	2				2	1	1
. 1	2			1	2	1		1.				2	2	2
2				ī		ī		$\bar{1}$				2	1	2
1				$\bar{2}$				1				1	2	$\bar{2}$
ī				_				2				ī	ī	- 1
. 2	ī	ĩ	32	$\overline{2}$	-ī	- 2	48	$\bar{2}$	ī	ĩ	64	2	$\tilde{2}$	-
	N1 2 1 1 2 1 1 2 1 Hi 2 1 1 1 1 1 1	N1 K2 2 2 2 1 1 1 1 1 2 1 2 2 1 2 High F 2 1 1 1 2 2 1 2 2 1 1 1 2 2 1 1 2 2 1 2 2 1 1 1 2 2	2 2 2 2 2 1 1 1 1 2 1 2 2 2 2 1 1 1 1 2 2 1 1 1 2 2 1 1 1 2 2 2 1 1 1 2 2 2 2 2 2 1 1 1 2 1 2 1 2 1 1 2 1 2 1 1 2 1 1 2 1 1 2 1	N1 K2 S1 17 2 2 2 18 2 1 1 19 1 1 2 20 1 1 1 2 20 1 1 2 22 2 2 1 23 1 2 2 24 High Fe Plot 2 1 2 25 1 1 1 26 2 2 1 27 1 2 2 28 2 2 2 28 2 2 2 29 1 1 2 30 1 2 1 31	N1 K2 S1 17 N1 2 2 2 18 1 2 1 1 19 2 1 1 2 20 2 1 1 1 21 2 2 1 2 22 1 2 2 2 1 2 2 2 2 2 4 High Fe Plot Lo 2 1 2 25 2 1 1 26 2 2 2 2 2 1 27 1 1 2 2 2 28 1 2 2 2 2 299 1 1 2 1 31 1	N1 K2 S1 17 N1 K2 2 2 2 2 18 1 1 1 1 1 2 20 2 2 1 1 1 1 21 2 1 2 1 2 2 2 1 2 2 2 1 1 2 22 1 2 2 2 1 2 2 2 1 2 2 2 1 1 1 1	N1 K2 S1 17 N1 K2 S1 2 2 2 18 1 1 2 2 1 1 19 2 1 1 1 1 2 20 2 2 2 1 1 1 2 2 2 2 2 1 1 2 2 2 1 2 2 2 2 1 2 3 2 2 1 High Fe Plot Low Fe 2 1 2 25 2 2 2 1 1 1 1 26 2 1 1 2 2 2 1 27 1 1 2 1 2 2 28 1 2 1 2 2 2 2 9 1 1 1 1 1 2 30 2 2 1 1 1 2 3 1 2 2	N1 K2 S1 17 N1 K2 S1 33 2 2 2 2 18 1 1 2 34 2 1 1 19 2 1 1 35 1 1 2 20 2 2 2 36 1 1 1 2 12 2 2 37 2 1 2 22 1 2 2 38 2 2 1 2 2 2 1 2 2 38 2 2 2 1 23 2 2 1 39 1 2 2 2 2 4 1 1 1 40 High Fe Plot Low Fe Plot 2 1 2 25 2 2 2 41 1 1 1 26 2 1 1 42 2 2 1 27 1 1 2 43 1 2 2 2 28 1 2 1 44 2 2 2 2 2 9 1 1 1 45 1 1 2 30 2 2 1 46 1 2 1 31 1 2 2 47	N1 K2 S1 17 N1 K2 S1 33 N1 2 2 2 2 18 1 1 2 34 1 2 1 1 19 2 1 1 35 2 1 1 2 20 2 2 2 36 2 1 1 1 2 1 2 1 2 37 1 2 1 2 22 1 2 2 38 2 2 2 1 23 2 2 1 39 1 1 2 2 2 2 1 23 2 2 1 39 1 1 2 2 2 2 1 1 1 40 2 High Fe Plot Low Fe Plot L 2 1 2 25 2 2 2 41 1 1 1 1 26 2 1 1 42 2 2 2 1 27 1 1 2 43 2 1 2 2 2 8 1 2 1 44 1 2 2 2 2 2 9 1 1 1 45 1 1 1 2 30 2 2 1 1 46 1 1 1 2 1 31 1 2 2 47 2	N1 K2 S1 17 N1 K2 S1 33 N1 K2 2 2 2 18 1 1 2 34 1 1 2 1 1 19 2 1 1 35 2 1 1 1 2 20 2 2 2 36 2 2 1 1 1 2 21 2 37 1 2 2 1 2 2 1 23 2 2 1 39 1 1 1 2 2 2 24 1 1 1 40 2 2 High Fe Plot Low Fe Plot Low F 2 1 2 25 2 2 2 41 1 1 1 1 26 2 1 1 44 1 2 2 2 2 2 8 1 2 1 44 1 2 2 2 2 2 2 9 1 1 1 45 1 1 1 1 2 30 2 2 1 46 1 2 1 1 1 2 30 2 2 1 46 1 2 1 2 1 31 1 2 2 47 2 2	N1 K2 S1 17 N1 K2 S1 33 N1 K2 S2 2 2 2 18 1 1 2 34 1 1 1 1 2 1 1 1 2 1 1 2 1 2 1 2 1 2 1	N1 K2 S1 17 N1 K2 S1 33 N1 K2 S2 49 2 2 2 18 1 1 2 34 1 1 1 50 2 1 1 1 19 2 1 1 35 2 1 2 51 1 1 2 20 2 2 2 36 2 2 1 52 1 1 1 21 2 1 2 37 1 2 1 53 2 1 2 22 1 23 2 2 1 39 1 1 2 55 1 2 2 1 23 2 2 1 39 1 1 2 55 1 2 2 2 2 4 1 1 1 40 2 2 2 56 High Fe Plot Low Fe Plot Low Fe Plot 2 1 2 25 2 2 2 41 1 1 1 57 1 1 1 26 2 1 1 2 43 2 1 2 59 1 2 2 2 2 8 1 2 1 44 1 2 2 60 2 2 2 2 2 2 9 1 1 1 45 1 1 2 61 1 1 2 30 2 2 1 46 1 2 1 62 1 2 1 2 1 2 30 2 2 1 46 1 2 1 62 1 2 1 31 1 2 2 663	N1 K2 S1 17 N1 K2 S1 33 N1 K2 S2 49 N1 2 2 2 2 18 1 1 2 34 1 1 1 50 2 2 1 1 1 19 2 1 1 35 2 1 2 51 2 1 1 2 20 2 2 2 36 2 2 1 52 1 1 1 1 2 1 2 1 2 37 1 2 1 53 2 2 1 2 22 1 2 2 2 38 2 1 1 54 2 2 1 2 22 1 2 2 2 38 2 1 1 54 2 2 2 1 2 3 2 2 1 39 1 1 2 55 1 1 2 2 2 2 4 1 1 1 1 40 2 2 2 56 1 High Fe Plot Low Fe Plot Low Fe Plot Hi 2 1 2 2 2 5 2 2 2 41 1 1 57 1 1 1 1 26 2 1 1 42 2 2 2 1 58 1 2 2 1 2 2 28 1 2 1 44 1 2 2 60 2 2 2 2 2 2 9 1 1 1 45 1 1 2 61 2 1 1 2 30 2 2 1 46 1 2 1 62 1 1 2 1 31 1 2 2 47 2 2 66 3 1	N1 K2 S1 17 N1 K2 S1 33 N1 K2 S2 49 N1 K2 2 2 2 18 1 1 2 34 1 1 1 50 2 1 2 1 1 1 2 2 1 1 1 2 2 2 2 2 1 1 51 2 2 1 1 1 1

^a Legend: N1=100 lb. nitrogen per acre; N2=500 lb. nitrogen per acre; K1=no potash; K2=300 lb. potash per acre; Fe1=4 per cent FeSO₄ applied as a fine mist monthly; Fe2=4 per cent FeSO₄ applied twice weekly; S1=no sterilization of the soil; S2=150 lb. per acre of chloropicrin injected into the soil before planting.

Test 1

This experiment was planted early in October of 1939. An infestation was made February 20 and 21, 1940. Infested immature fruits were used and a section of the rind placed in the heart of each plant, as in nitrogen test 3. On February 28, 1940, establishment of vigorous mealybug colonies seemed to be general, and one month after infestation the plots were sprayed with an oil emulsion to eliminate the mealybugs. The final wilt readings on these plots were made on July 24, 1940, and the data are summarized in table 2.

Test 2

This experiment was an exact duplicate of the first and was in exactly the same area with only a short 3-month intercycle period between clearing the plots of the plants from test 1 and planting those of test 2. The purpose of this arrangement was to determine whether the position of wilted plants would remain the same as in test 1. The plants were planted October 2, 1940, and infested with mealybugs on February 25, 1941. Wilt readings were completed on July 30, 1941, and are summarized with those of test 1 in table 2, for comparison with results from test 1.

Test 2a

This test was accomplished by reinfesting one half the plots of test 2, on July 30, 1941, when the wilt readings for test 2 were completed, and the plants which had shown wilt were recovering. The results of this reinfestation, shown in tables 3 and 4, reveal a curious significance in the differences between certain of the treatments but only in the case of those plants which had recovered from severe wilt. Plants in the low N plots wilted a second time in numbers significantly greater than in the high N plots, and, to a lesser degree, significantly greater in the no-chloropicrin plots than in those treated with chloropicrin (Table 4).

TABLE 2.—Factorial tests 1 and 2. Experiments on the effect of nitrogen, potash, iron, and soil sterilization on susceptibility, the plots being factorially arranged. Table showing number of plants wilted in tests 1 and 2. Plants infested when five months old. Each plot included approximately 138 plants with four replications of each treatment

					Test	1: I	nfeste	ed 1940		7	est :	2: Ir	feste	d 1941	
						Plant	s wilt	ed		Plants wilted					
'1	Treatment		 Replicatio		tions			Per	Re	Replications				Per	
			,1	2	3	4	Total	cent	1	2	3	4	Total	cent	
N1	K2	S1	F1	 102	96	82	113	393	71	106	95	129	96	426	77
2	2	2	1	93	61	91	88	333	60	78	78	116	102	374	68
2	1	1	1	95	106	115	88	404	73	123	101	109	100	433	78
1	1	2	1	63	84	98	67	312	57	97	99	128	123	447	81
1	1	1	1	74	118	68	72	332	60	88	109	82	82	361	65
2	1	2	1	78	69	106	77	330	60	109	106	107	107	429	78
2	2	1	1	104	96	114	93	407	74	115	131	78	96	420	76
1	2	2	1	91	70	116	70	347	63	. 96	130	105	68	399	72
2	1	2	2	68	82	118	92	360	65	- 88	109	119	129	445	81
1	- 1	1	2	100	89	72	78	339	61	92	79	97	106	-374	68
2	2	1	2	93	94	88	75	350	63	131	92	132	89	444	.80
1		2	2	112	73	89	65	339	61	105	99	111	129	445	80
2	2	2	2	101	72	72	99	344	62	122	91	55	116	384	70
1	1	2	2	73	112	73	88	346	63	126	80	75	68	349	63
1	2	1	2	65	84	76	99	324	58	122	77	113	62	374	68
2	1	1	2	99	92	60	116	367	66	111	124	94	104	433	78

TABLE 3.—Results of reinfesting plants in factorial test 2 which had recovered from severe wilt, initial symptoms only, or had remained symptom-free following the first infestation

Treatment		plants reinf n each class	ested	Percentage plants wilted in each class						
group	Symptom- free	Initial stages only	Severe wilt	Symptom- free	Initial stages only	Severe wilt				
All	1,129	278	410	34.7	33.4	30.0				
Low N		113	232	33.5	38.1	35.8				
High N	478	165	178	36.4	30.3	22.5				
No chloropicrin	530	161	177	34.1	34.6	35.5				
Chloropicrin	599	117	233	35.2	32.4	25.7				
	ار سے سے	93	216	34.4	37.6	26.8				
Low K	554	90								
		185	194	34.9	31.3	33.5				
Low K High K Low Fe	575									

TABLE 4.—Statistical treatment of data from table 3. Underlined figures are significant

Dient types	χ² valu	χ^2 values from table				
Plant types - reinfested	Lo	w vs. hig	;h	Chloropicrin	D 0.05	TD 0.01
	N	K	Fe	+ vs	P = 0.05	P = 0.01
Healthy	1.033	0.029	2.783	0.143	3.841	6.635
Initial stages only	1.810	1.097	0.088	0.086	3.841	6.635
Severe wilt	8.489	2.154	2.731	4.640	3.841	6.635

Test 3

This test was laid out in precisely the same manner as tests 1 and 2, the only difference being that the plots were not so large, each plot containing about 85 plants instead of about 140. The plants grew without mealybug infestation until they were $9\frac{1}{2}$ months old, when they were infested by the fruit-rind method. By this time, very great differences in growth status had developed throughout the plots. The results of this test are in table 5. The low percentage of plants wilting, when compared with tests 1 and 2 is further confirmation of the reduced susceptibility of older plants.

Test 4

This was a duplicate of test 3 as far as factorial arrangement, size of plots, age of plants, and time of infestation were concerned, except that only

TABLE 5.—Factorial tests 3 and 4. Experiments on the effect of nitrogen, potash, iron, and soil sterilization on susceptibility, the plots being factorially arranged. Table showing number of plants wilted in test 3. Plants infested when $9\frac{1}{2}$ months old. Each plot included approximately 85 plants with four replications for each treatment in test 3, and 2 replications in test 4

					Te	st 3:]	Infest	ed 1941				T	est 4:]	Infested	1942		
m						Plar	nts wil	ted			Plants wilted						
1.	Treatment]	Total		Per	Re	plic	ations	<i>m</i>	Per					
				1	2	3	4		cent		1	2	Total	cent			
N1 -	K_2	si I	1	5	20	21	6	52		16		24	26	50	29		
2	2	2	1	3	8	26	8	45		13		10	11	21	12		
2	1	1	1	10	19	22	2	53		16		31	18	49	28		
1	1	2	1	29	30	22	32	113		33		20	24	44	25		
1	1	1	1	39	25	24	30	118		35		28	46	74	43		
2	1	2	1	. 31	12	32	20	95		28		30	33	63	-36		
2	2	1	1	13	37	25	24	99		29		40	18	58	33		
	2	2	1	12	36	21	34	103		30		40	37	77	45		
2	1.	2	2	13	20	6	11	50		14		16	17	33	20		
1	1	1	2	19	13	12	34	78		23		41	6	47	. 27		
2	2	1	2	19	33	17	21	90		26		24	13	37	22		
1	2	2	2	15	28	6	15	64		19		29	16	45	26		
- 2	2	2	2	21	24	33	12	90		26		12	39	51	30		
1	1.	2	2	40	12	23	20	95		28		13	17	30	1.8		
1	2	1	2	34	8	18	24	84		25		1	13	14			
: 2	1	1	2	13	10	21	22	66		20		18	36	54	32		

one-half the total number of plots were infested. Wilt following the infestation in this test is shown along with the data from test 3, in table 5.

PHYTOPATHOLOGY

DISCUSSION

When plants are infested within three months of planting, differences in growth status due to fertilizer level are apparent but may not have had sufficient opportunity to affect susceptibility significantly. From six months on, however, the variations in fertilizer practice used in these experiments resulted in extreme variation in plant growth. The low-nitrogen plants were small, yellow green, with tough narrow leaves, while at the other extreme were the large, dark green, succulent, wide-leaved plants in the high N plot, especially where the soil was partially sterilized before planting.

In spite of these extremes, no striking relationship exists between plant growth status and susceptibility to wilt. The detailed data were subjected to analysis of variance with the result that significance (p = .05 or odds of 19 to 1) was shown in nitrogen test 2 to the extent that wilt was less in the 1,000-lb. N plot than in either the 0- or 500-lb. N plots. In potash test 1, there was significantly more wilt in the 300-lb. plot than in either the 0- or 600-lb. plots. Since other experiments including these same variables failed to show similar results, too much importance cannot be attached to these significances. Variation between numbers of plants wilted in plots of similar treatment are so great that very great differences between treatments would be necessary to show significance. This variation between plots is believed due to the localization of wilt in small areas within the field. This phenomenon is discussed further in the concurrent paper, but it is clear that it has overwhelmed any effect on susceptibility that the fertilizer treatments might have had.

When the four factorial experiments were analyzed, statistical significance was shown in test 3, not between the two iron treatments, but in the interactions between iron and other experimental variables. The factorial experiments were on such an extensive scale that this result cannot be disregarded entirely, even though none of the other experiments showed a similar result; but to account for it, a very delicate balance must be postulated between plant nutrition and photosynthetic activity which in some manner affects susceptibility.

Test 2a, in which plants were infested for a second time, shows the only striking relationship between treatments and wilt incidence. Plants which had had severe wilt symptoms following the first infestation but had recovered in typical fashion, wilted a second time following reinfestation by mealybugs in significantly greater numbers (p = 0.01 or odds of 99 to 1) in the low N plots than in the high N plots, and to a lesser significant degree (p = 0.05 or odds of 19 to 1) in greater numbers in the no-chloropicrin plots (Tables 3 and 4). Whatever the correct explanation for these results may be, it is clear that plants which were recovering from severe wilt were rendered more susceptible to wilt a second time under conditions adverse to

growth. From the standpoint of mealybug wilt control, the results obtained when young ration suckers were infested, make it clear that wide variations in fertilizer application to mother plants failed to affect the extremely susceptible young suckers. Since this period in the life of a pineapple field is the most critical one with respect to increase in mealybug wilt incidence, there is little hope of modifying the severity of mealybug wilt by agronomic methods.

SUMMARY

Pineapple plants grown at various levels of N, P, K, Ca, and Fe develop with extreme variation in growth status, which was accentuated by partial soil sterilization with chloropicrin. These plants were infested with mealy-bugs and the resulting mealybug wilt recorded, but the differences in fertilizer application failed to affect the susceptibility of the plants to wilt. High N application appeared to reduce susceptibility, but in one test only.

The most significant result was in the reinfestation of plants that were recovering from severe wilt. Adverse growth conditions increased the susceptibility of these plants to a second wilting.

PINEAPPLE RESEARCH INSTITUTE, UNIVERSITY OF HAWAII, HONOLULU, HAWAII.

A CERCOSPORA LEAFSPOT OF CULTIVATED PHYSOSTEGIA¹

JENKINS WILBERT Α.

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INTRODUCTION

During August, 1943, a destructive leaf spot of cultivated Physostegia virginiana Benth, came to the writer's attention. The disease was so severe and the resulting defoliation so acute that an effort was made to identify the nathogen. After it became apparent that the disease was caused by an undescribed species of Cercospora, a study of the life cycle of the pathogen was undertaken. A portion of the results form the basis of this report.

SUSCEPTS AND RANGE

Field observations have been confined to a small area in the vicinity of Chatham, Virginia, where the disease is prevalent. Nothing is known of the general distribution of the disease, nor is the disease apparently known on wild relatives of the species in question.² Somewhat closely related species of the mint family in this area show no evidence of susceptibility to the disease.

SYMPTOMATOLOGY

The earliest observed symptoms appeared during the middle to latter part of June on the lowermost leaves. Typical symptoms persist on these lower leaves, scarcely noticeable to the casual observer until the latter part of July, but meanwhile progressing slowly up the plant from leaf to leaf. Very few symptoms have been seen on any portion of the plant other than the foliage, though when the disease becomes epiphytotic during August it is not uncommon to find lesions on the flower bracts.

Lesions vary from one to several centimeters, are at first pale vellow with slightly darker centers, and generally appear first on the upper leaf surface. A typical halo, usually rather consistently associated with such lesions, is absent. As the lesion develops, its color approaches brick-red and finally becomes pale to dark brown. Meanwhile, the lesion spreads rather uniformly over the leaf surface, finally becoming slightly depressed and dry (Fig. 1, A). On the lower leaf surface, a generalized pale color soon marks the limits of the lesion during early developmental stages. Later, color changes occur within the lesions, but they are never so intense as those on the upper leaf surface, nor are the limits of the lesions ever so well defined (Fig. 1, B). Through coalescence of lesions, most of the surface of affected leaves is ultimately involved; the leaves may curl or there may be more or less complete defoliation.

1 Published with the approval of the Director, Virginia Agricultural Experiment Station, as Scientific Paper No. 123 from the Section of Botany and Plant Pathology.

2 After this report was submitted for publication, correspondence and exchange of specimens with Dr. B. B. Higgins established the presence of this disease in the vicinity of Griffin, Georgia, where it was destructive in 1941.

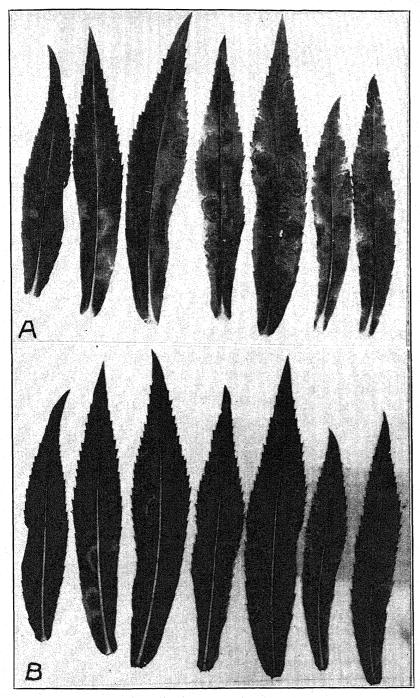


Fig. 1. Photographs showing a graduated series of symptoms of Cercospora leaf spot of leaves of cultivated *Physostegia virginiana* Benth. (A) Upper leaf surface; (B) lower leaf surface. The semblance of a halo about certain lesions is due to the photographic filter. (Original photographs $\times \frac{1}{2}$.)

Conidiophore stromata appear as discrete, tawny tufts within the older lesions. Normally, sporulation is confined to the upper surface of the lesions. With age and at high humidity sporulation often becomes amphigenous.

ETIOLOGY

Being unable to determine the specific identity of the pathogen with the literature at hand, the author sent specimens to Professors F. A. Wolf and Charles Chupp, of Duke University and Cornell University, respectively. Professor Wolf reported that no species of Cercospora on Physostegia, nor on its synonym, Dracocephalum, was listed in the literature available to him and that the species appeared to be undescribed. Professor Chupp stated that the species was new according to his keys and that it did not resemble any other of the known forms on the Labiatiae. He also stated that he was filing the specimens in his herbarium as Cercospora Physostegiae W. A. Jenkins, awaiting my description of the species. Because we now know that the pathogen possesses a normal and typical life cycle, including spermogonia and perithecia, typical of species of Mycosphaerella heretofore described (1, 2, 3, 4, 5), it will hereafter be referred to as Mycosphaerella Physostegiae n. sp.

DEVELOPMENTAL MORPHOLOGY

Results of inoculations under controlled conditions indicate that infection may occur through either leaf surface, though more uniformly through the lower one, and that penetrations occur through stomata or directly through epidermal walls. Infection hyphae appear to be intercellular at first, but soon an intracellular relationship is established. Haustoria, in the accepted sense of the term, were not seen, though the characteristic coiling of the hyphae within parasitized cells, approximating a loose stromatic formation, might readily be assumed to perform haustorial functions. Conidiophores originate from subcuticular or intraepidermal hyphae and begin the production of conidia soon after emergence, long before basal anastomosis with attendant stroma formation is evident (Fig. 2, A). With age they assume the characteristics of typical conidiophores of *Cercospora*.

The conidia, en masse, appear bluish-grey; singly they are hyaline to subhyaline. They are slender, more or less curved, blunt on the basal end, tapering to subacute on the distal end, with a tendency toward two oil droplets within each cell of the older spores, 1–6 septate, and 17–112 \times 2.28–6.08 μ (Fig. 2, B). Under average field conditions, the spores average 48.5 \times 4.24 μ , but those produced at high humidity may be as long as 112 μ . Length and septation of conidia, as with other species of Cercospora studied, vary with relative humidity in the environment during their formation, but width remains relatively constant. The spores germinate readily in from 3 to 8 hours on tap-water agar when the moisture and oxygen balance are favorable. Germ tubes emerge from terminal cells at either or both ends of the spore, and often from other cells, as well (Fig. 2, C).

During late September and early October, the saprophytic cyclic develop-

ment is initiated within the leaves that have fallen from the plants. Some evidence of such development may also be noted on foliage still attached, but it is evident that such leaves are essentially senescent, so that one might readily assume the relationship to be a saprophytic one. Spermogonia and perithecia are initiated concurrently, either within conidiophore bases or independently of such structures. In the latter instances, spermogonial and

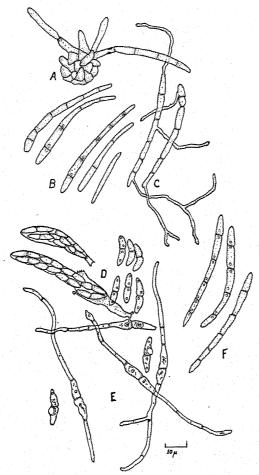


Fig. 2. Spore forms of Mycosphaerella Physostegiae n. sp. A. Young conidial stroma before development of a pronounced basal stroma. B, C, F. Conidia: B, from field material; C, showing method of germination; D, from ascospore culture on potato-dextrose agar: D, E. Asci and ascospores: D, an ascus intact, a mature ascus with ruptured outer membrane, and recently discharged ascospores; E, germinating ascospores. (All sketches drawn to scale, original with aid of a camera lucida, ×1,200 dia.)

perithecial fundaments originate from subcuticular, intraepidermal, or rarely, from subepidermal stroma. Owing, perhaps, to fluctuating climatic conditions, it is not unusual to find fertile conidiophores developing from the exterior surfaces of both spermogonia and perithecia.

Very young spermogonia and perithecial fundaments are indistinguish-

able one from the other, but with limited development they may be readily separated on the basis of content. As with other species of *Mycosphaerella* studied critically (1, 2, 3), the perithecial fundaments soon produce one to several archicarps, while the spermogonia develop spermatial mother cells.

The spermogonia originate about the periphery of old lesions or within them and are about equally abundant on both leaf surfaces. The details of development are essentially identical with those reported for other species of Mycosphaerella. At maturity, the spermatia occur in groups of 3 or 4 within the spermatiferous cells and are liberated through sterigma-like processes from them. Mature spermogonia are $27-60 \times 27-56 \mu$. Mature spermatia are rod-shaped, uninucleate, hyaline, and $1.5-4 \times 0.5-0.8 \mu$.

Perithecial fundaments originate concurrently with the spermogonia. Their distribution is also the same, but they soon produce one to several deep-staining archicarps, each with an elongate trichogyne and an enlarged, uninucleate basal cell. Cytological details of perithecial development thus far observed differ in no important respects from those already published for other species of the genus (1, 2, 3). The perithecia are rostrate when young and at maturity are $47-95 \times 40-75 \mu$.

The perithecia of Mycosphaerella Physostegiae develop slowly as compared with several other known species, mature spores in the field having first been found on June 9, 1944. Sectioned material confirmed the week of June 9 as being the time of earliest maturation of spores. This delay in perithecial maturity is occasioned by the very slow development of the ascogonia following spermatization. The asci do not mature at one and the same time within a given perithecium so that perithecia function as the source of primary inoculum over a considerable period, depending on the frequency of moisture. In addition, conidia are produced abundantly from the surfaces of both sterile and fertile stromatic masses, beginning as early as May 12 in 1944, and continuing on concurrently with ascospore maturation and discharge.

DEVELOPMENT IN CULTURE

Conidial isolates were obtained by the streak method on hard tap-water agar, while ascospore isolates were obtained by causing the ascospores to discharge upward and cling to the surface of tap-water agar in inverted Petri dishes. Mature spores were measured in crushed mounts and soon after their discharge into the Petri plates. Under such conditions the ascospores are hyaline to subhyaline, guttulate when young, straight to slightly curved, bicellular, and $11.6-17.8 \times 2-3.8 \,\mu$. The asci are bitunicate and the ascospores become biseriately arranged prior to discharge (Fig. 2, D). Because of its structure and the dynamics attendant on its function, a mature ascus is difficult to measure for length; nor, in the writer's opinion, is such a measurement of taxonomic value (Fig. 2, D).

Spores of both types germinate readily within 3 to 8 hours and produce visible growth within 3 days (Fig. 2, C-E). Cultures from conidia and ascospores on potato-dextrose agar are identical in all measurable respects.

When first visible, the mycelium is pale grey to almost white, but the portion of the colony adjacent to the medium finally becomes deep olive to almost black. The colonies are pulvinate from the beginning and after 5 to 6 days indications of a stroma appear adjacent to the surface of the medium, particularly in isolates from ascospores. Isolates from both sources produce conidia abundantly within 3 to 5 days and the conidia are indistinguishable from each other and from those produced under similar conditions of temperature and moisture on field material (Fig. 2, B, F). Stromatic masses were produced in old cultures from both sources, but recognizable spermogonia and perithecia were not observed in any of the cultures.

Both conidia and ascospores were used as inoculum. The conidia were obtained from fresh field material as well as from conidial and ascospore isolates. In all cases resulting infections were identical and occurred within 10 to 21 days following inoculation.

The spore forms discussed constitute a pleomorphic cycle of one and the same organism.

TAXONOMY

The form and development of the perithecia, the asci produced in fascicles, the absence of paraphyses, and the bicellular, hyaline spores are characteristic of the genus Mycosphaerella Johans. The fungus is designated Mycosphaerella Physostegiae n. sp., with the following diagnosis.

Mycosphaerella Physostegiae n. sp.

Syn: Cercospora Physostegiae W. A. Jenkins (In Herb.)

Perithecia numerous, mostly in lesions, amphigenous, partly embedded in host tissue, erumpent, ovate to nearly globose, beaked prior to maturity, $47-95\times40-75\,\mu$, black, ostiolum papillate when mature; asci club-shape, short stipitate, fasciculate, $36-40\times10-20\,\mu$, aparaphysate, bitunicate, eight-spored; spores biseriate to imperfectly uniseriate in the ascus, bicellular, straight to slightly curved, hyaline to subhyaline, guttulate, $11.6-17.8\times2-3.8\,\mu$.

Hab. In overwintered lesions produced by the conidial stage on leaves of *Physostegia virginiana* Benth. (cultivated), Chatham, Virginia, maturing during early June.

Spermogonia: Numerous, in and along the margins of lesions produced by the conidial stage, ovate to globose, black, amphigenous, embedded in leaf tissue but later erumpent, ostiolate, $27-60 \times 27-56 \,\mu$; spermatia small, rod-shaped, hyaline, $1.5-4 \times 0.5-0.8 \,\mu$, arising endogenously, usually in fours within spermatiferous cells and liberated through sterigma-like processes.

Hab. On recently fallen leaves, maturing from October through February.

Conidial stage: Lesions circular to indefinite, often confluent, varying from 1 mm. to several cm., pale to dark brown, confined to foliage; conidiophores becoming amphigenous with age, base becoming stromatic; fasciculate, geniculate, pigmented at base, continuous to one- to several-septate, mostly short; conidia hyaline to subhyaline, slender, cylindrical, club-shaped, blunt on basal end, tapering to subacute on distal end, curved, $17-112\times2.28-6.08\,\mu$ (mostly $48-60\times3.8-4.2\,\mu$), 1-6 septate, guttulate, length and septation influenced by humidity.

Hab. Parasitic on leaves of cultivated Physostegia virginiana Benth., causing leaf

spots and contributing to premature defoliation. -

Peritheciis dense aggregatis, plerumque in maculis, amphigenis, semi-immersis, punctiformibus, ovatis vel globatis, rostratis cum immaturis, $47-95\times40-75\,\mu$, nigris, ostiolis papillato praeditis; ascis cylindraceis elavatis, brevisme stipitatis, fasciculatis, aparaphysatis, bitunicatis, octosporis, $36-40\times10-20\,\mu$; sporiidis biseriatis vel sub-uniseriatis, bicellularibus, vix curvatis, hyalinus vel sub-hyalinis, guttulatis, $11.6-17.8\times2-3.8\,\mu$.

Hab. in foliis dejectis Physostegia virginianae, Chatham, Virginia.

Spermogoniis autumno efformatis, dense aggregatis, plerumque in maculis et marginatis, ovatis vel globatis, nigris, amphigenis, innatoerumpentibus, punctiformibus, $27-60\times27-56\,\mu$; spermatiis bacillaribus, hyalinis, $1.5-4\times0.5-0.8\,\mu$.

Hab. in foliis dejectis Physostegia virginianae.

Statu conidico in maculis circularibus v. irregularibus, saepe confluentibus, ocraceis v. rubro-ferrugineis, foliis efformato; hyphis fertilibus amphigenis, a stromate orientibus, fascilatis, geniculatis, hyalinis v. dilute olivaceis-grieseis, continuis v. pluriseptatis, brevisme; conidiis hyalinis v. sub-hyalinis, attenuatis cylindraceis obclavatis v. sub-clavatis, curvulis, $17-112\times2.28-6.08\,\mu$ (plerumque $48-60\times3.8-4.2\,\mu$), 1-6 septatis, guttulatis.

Hab. in foliis vivis Physostegia virginianae.

For the convenience of plant pathologists and mycologists, materials have been deposited in the following herbaria: Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture; Plant Pathology Department, Cornell University, Ithaca, N. Y., the Herbarium of the Georgia Experiment Station, Experiment, Georgia.

CONTROL

Results from a single season's work indicate that Cercospora leaf spot of cultivated Physostegia virginiana Benth. may be rather effectively controlled by simply removing the fallen leaves from beneath the planting any time before mid-April of the following year. Since the plantings in Virginia are propagated by rhizomes, it would be impractical to turn under the fallen The late maturity of the perithecia greatly simplifies removal and destruction of the fallen leaves, preferably by burning, as a control measure.

SUMMARY

The symptomatology and etiology of a destructive leaf spot of cultivated Physostegia virginiana Benth. have been studied over a complete season. Symptoms first appear during June and July on the lowermost leaves, but the disease does not become epiphytotic until August. The lesions appear pale yellow at first with dark centers, but develop through shades of brickred to a final pale to dark brown and then enlarge until often most of the leaf surface is involved.

The pathogen, heretofore undescribed in any stage of development, produced a hyphomycetous conidial stage characteristic of the form genus Cercospora, also spermogonia and perithecia. The perfect stage is herein described as Mycosphaerella Physostegiae n. sp.

A single season's observations indicate that Cercospora leaf spot of cultivated Physostegia virginiana Benth, may be controlled by removing and burning the cast leaves any time between leaf fall and mid-April of the following season.

VIRGINIA AGRICULTURAL EXPERIMENT STATION, TOBACCO RESEARCH LABORATORY, CHATHAM, VIRGINIA.

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VARIETAL RESISTANCE AND SUSCEPTIBILITY OF WHEAT TO FLAG SMUT (UROCYSTIS TRITICI KÖERN.). IV. FURTHER STUDIES ON PHYSIOLOGIC SPECIALIZATION IN UROCYSTIS TRITICI KÖERN.¹

T. F. Yu, H. R. WANG, AND C. T. FANG²
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INTRODUCTION

In an earlier paper (4), the senior writer and his co-workers reported 5 physiologic races of *Urocystis tritici* Köern. from China. Since the publication of that paper, additional collections of the smut from various parts of China have been tested and 7 new races have been described and numbered, thus bringing the number of known races to 12.

MATERIAL AND METHODS

In addition to the 5 physiologic races of *Urocystis tritici* reported in 1936 (4), 37 smut collections were obtained from common wheat (*Triticum vulgare*), and one collection on a poulard wheat (*T. turgidum*) was obtained and used in 1942–43.

For differential hosts, 17 varieties of wheat including Nanking Nos. 716, 793, 795, and 799, which had been used in the previous studies, were chosen. They are outstanding for certain characters and representative of some wheat growing regions of China. In the final analysis of the results, however, only 5 of them have been selected as differential hosts: Nanking No. 716, developed by the Department of Plant Pathology, Nanking University, for its high yield and flag smut resistance (3); Grassland, a high-yielding variety in Kweichow, originated at Kweichow Provincial Agricultural Experiment Station; Ngochen, reported to have originated from the commercial wheat grown in Ngochen; Tsing Hua No. 1932, originated by selection from commercial wheat in Yunnan; and Tsing Hua No. 559, a poulard wheat (Triticum turgidum), commonly grown in Central South China. With the exception of the last one, all are common wheat (T. vulgare).

All seed was disinfected in a formaldehyde solution (1:320) for 1 hour and washed and dried before sowing. A measured quantity of spores was shaken with the seed until the seed was completely covered with spores. A complete series of wheat hosts was so inoculated for each collection of smut. The inoculated seed in each year's test was planted at the rate of 3 grams to the 3-foot row. Each plot consisted of three 3-foot rows for each variety,

¹ Paper No. 21 from the Division of Plant Pathology, The Institute of Agricultural Research, National Tsing Hua University, Kunming, Yunnan, China.

Research, National Tsing Hua University, Kunming, Yunnan, China.

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systematically distributed and replicated in 4 series. The check plots of uninoculated seed of each variety so treated produced smut-free plants. The smut percentages obtained were based on counts of the total number of culms per plot. As there was very little difference in the percentages of smut in the replicated plots, only the averages are recorded.

In the analysis of data, three infection classes were arbitrarily established: 0-5 per cent infection = Resistant class (R); 5.1-20 per cent infection = Intermediate class (I); and 20.1-100 per cent infection = Susceptible class (S).

RESULTS

The experiments were at Kunming, Yunnan, China, from 1939 to 1943, inclusive. The 5 physiologic races previously reported, together with 37 additional collections of the smut, were tested on 17 varieties of common wheat. Twenty-one of these 37 smut collections produced consistent results and have been identified. The rest of them are reserved for further studies. Of the 17 varieties of common wheat, 4, namely, T. H. 1932, N. 716, Ngochen, and Grassland, were retained as differential hosts. The others, on which results had been inconsistent, were discarded. On the basis of the reaction of these 4 varieties of wheat, it was possible to differentiate the smut collections into 11 physiologic races. Five of them had been previously identified and the remaining 6 are recorded as new races. The annual and average percentages of smut produced by each of the smut collections, in each of the 4 years tested, are given in table 1.

There are sufficiently great differences in the virulence of the collections of smut to justify the conclusion that they are different physiologic races. Races 4 and 5 are differentiated from the rest by the intermediate reaction of N. 716. T. H. No. 1932 is resistant to race 4 but is intermediate in its reaction to race 5, which is the only race that can infect all of the differential hosts. Races 10 and 11 are distinct from other races by their virulence on Grassland and are differentiated by the difference in reaction of Ngochen and T. H. 1932 to them. The identity of race 2 is based on the susceptibility of T. H. No. 1932 to it, that of race 7 on the susceptibility of Ngochen. Race 6 is the only race to which all four differential hosts are resistant. It is similar to races 1 and 9.

The variety T. H. No. 3929 was originally selected as the susceptible check. The high percentages of smut produced on it indicate that the environmental conditions of the tests were favorable for smut infection. This variety is completely susceptible to the 11 physiologic races (Table 1).

In the course of breeding for flag smut resistance in both the common and poulard wheats, about 300 selections of the latter were inoculated with a mixed inoculum of the smut each year since the fall of 1938. In 5 years, none of these poulard wheat selections had ever been smutted. It was thus once believed that the poulard wheat might be immune from the disease. However, in the spring of 1942, an additional collection of flag smut from a commercial field of poulard wheat in Chengkung, Yunnan, was used to in-

TABLE 1.—Average percentages of flag smut obtained in 5 varieties of common wheat inoculated separately with 21 collections and grouped into 11 physiologic races of Urocystis tritici at Kunming, Yunnan, China, 1940–1943

TTt	77		Race 1	, Collec	tion No.	_		Race 2,	Collecti	on No.	<u>-</u>		Race 3	, Collect	tion No.	_
Host	Year	la	5	6	10	19	2a	11	12	20	21	3a	13	14	16	18
		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
T. H. No.	1940	2.1	1.4	2.4	- 0		22.6	11.0		22.9	10.1	$0.0 \\ 3.4$	2.6	3.1	2.3	0.0
1932	$1941 \\ 1942$	0.0	0.0	$0.0 \\ 0.5$	$\frac{5.6}{0.0}$	$\frac{3.2}{0.5}$	$18.3 \\ 17.5$	$\frac{11.3}{39.3}$	$21.1 \\ 43.9$	34.1	$19.1 \\ 41.5$	$\frac{3.4}{2.1}$	0.9	1.8	4.3	0.0
	1943	0.0	0.0	2.0	2.3	0.8	26.1	18.4	13.5	25.7	24.2	1.7	0.0	0.5	0.6	0.5
	Av.	0.5	0.5	1.2	2.6	1.5	21.1	23.1	26.2	27.6	28.2	1.8	1.2	1.8	2.3	0.1
N. 716	1940	0.0	0.0	0.0			0.6					2,2				
	1941	0.0	0.0	0.0	0.0	0.0	1.1	0.3	$\frac{3.1}{2}$	0.5	5.4	0.6	3.6	3.7	0.0	0.0
	1942	0.0	2.3	0.0	0.3	0.0	1.4	1.6	7.8	$0.0 \\ 4.9$	$\frac{5.2}{1.7}$	$\begin{array}{c} 3.4 \\ 1.5 \end{array}$	$\frac{7.4}{1.8}$	$\frac{8.1}{2.0}$	$\frac{2.7}{4.1}$	2.3
	1943	3.2	0.7	0.0	0.0	0.0	0.0	0.6	0.0	4.9	1.7	1.0	1.0	4.0	7.1	U.\
	Av.	0.8	0.8	0.0	0.1	0.0	0.8	0.8	3.6	1.8	4.1	1.9	4.3	4.6	2.3	0.
Ngochen	1940	6.3	0.0	5.2			1.2					10.2				
	1941	0.4	0.0	11.4	5.6	7.3	0.3	0.0	0.5	7.1	2.6	6.7	8.2	7.0	3.5°	4.8
	1942	9.1	15.7	1.7	9.7	4.1	0.6	9.1	2.9	4.2	7.7	13.3	$\begin{array}{c} 13.6 \\ 7.3 \end{array}$	$14.5 \\ 13.4$	$7.0 \\ 4.9$	9. 7.
	1943	7.8	6.3	3.7	0.3	4.0	1.9	4.0	0.1	3.0	1.9	4.9				
	Av.	5.9	5.5	5.5	5.2	5.1	1.0	3.4	1.1	4.8	4.1	8.8	9.7	11.6	5.1	6.
Grassland	1940	3.1	4.4	0.0	************		0.7				**********	9.6				,
	1941	0.0	3.7	0.0	2.7	0.0	2.4	2.9	0.3	8.1	1.8	8.3	8.2	7.4	7.3	5.0
	1942	3.4	9.2	0.0	4.0	2.2	4.6	4.0	1.4	0.7	6.1	2.5	17.1	20.6	9.2	3.8
	1943	1.5	1.0	0.5	0.0	0.0	1.9	1.0	0.0	1.3	6.0	4.6	19.7	16.0	16.7	6.
	Av.	2.0	4.6	0.1	2.2	0.7	2.4	2.6	0.1	3.4	4.6	6.4	15.0	14.1	11.1	5.5
Т. Н. No.	1940	18.1	52.7	31.5			37.2					33.7				
3929	1941	61.3	38.7	25.8	36.5	47.3	48.6	48.9	33.7	52.9	42.1	28.0	29.7	55.1	29.6	18.
	1942	52.4	71.6	68.1	25.7	62.2	41.9	73.3	75.8	57.1	63.0	41.3	50.9	43.0	51.3	29.
	1943	31.4	68.0	67.1	42.8	60.4	49.3	66.2	64.2	55.8	48.5	22.6	78.2	67.0	43.3	32.4
	Av.	40.8	57.8	48.1	31.9	56.8	44.2	69.6	57.9	57.4	51.1	31.4	52.9	55.1	48.1	26.8

TABLE 1.—(Continued)

\mathbf{Host}	Year		ce 4, No. —	Race 5, Coll. No. —		se 6, No. —	Race 7, Coll. No. —	Race 8, Coll. No. —	Race 9, Coll. No. —	Race Coll. I		Race 11 Coll. No.
			2	5a	8	17	4	1	3	9	15	7
		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	$Pct. \ 3.8$
T. H. No.	1940	0.0	1.1	9.9	0.0		2.9	7.2	0.0			1.5
	1941	1.7	0.0	11.8	0.0	3.4	0.7	13.6	0.0	5.2	3.2	
1932	1942	0.0	3.4	14.7	0.0	0.0	3.3	22.7	0.0	5.9	5.0	9.9
	1942	0.9	1.4	6.5	0.0	. 1.5	0.3	9.8	0.3	0.0	5.6	5.7
	Av.	0.7	1.5	10.7	0.0	1.6	1.8	13.3	0.1	3.7	4.6	5.2
	7010	П.С	5.5	14.3	0.0		1.5	0.0	0.0			2.8
N. 716	1940	7.6		17.5	0.0	0.0	0.0	0.0	0.0	6.8	5.5	6.0
	1941	11.3	10.1		1.4	1.4	3.1	3.2	0.4	5.3	3.9	6.5
	1942	8.8	9.2	8.8	0.6	0.0	0.4	0.0	0.5	1.6	2.8	3.1
	1943	10.1	7.4	10.1	0.0					1.0	4.1	4.6
	Av.	12.7	8.1	12.7	0.5	0.5	1.3	0.8	0.2	4.6	4.1	
			F.0	1.3	5.2		22.1	7.6	4.2	***********	***********	4.9
Ngochen	1940	5.9	7.2	$\begin{array}{c} \textbf{1.5} \\ \textbf{4.5} \end{array}$	5.6	5.2	36.5	18.8	1.9	6.7	6.1	3.6
**************************************	1941	3.0	8.9			5.5	15.0	21.3	2.5	13.5	12.4	8.2
	1942	8.3	3.6	10.7	3.9	$\frac{5.5}{2.9}$	19.0	5.4	1.9	7.0	9.0	0.7
	1943	7.2	7.3	5.2	1.4	∠.9	19.0					
	Av.	6.1	7.0	5.4	4.1	4.5	23.2	18.3	2.4	9.1	9.2	4.3
G	1940	5.5	7.6	9.2	0.0		0.0	0.0	8.4			17.2
Grassland	1941	12.1	10.0	1.3	3.3	1.4	0.0	0.0	16.2	19.9	20.7	23.8
	$1941 \\ 1942$	6.4	12.2	7.9	9.4	0.0	1.6	2.3	23.5	18.4	25.1	15.8
	1942	4.0	9.6	3.5	0.0	4.2	0.0	0.0	9.8	24.4	22.3	26.8
	Av.	7.0	9.9	5.5	3.2	1.3	0.4	0.6	14.5	20.9	22.7	20.8
	1010	41.7	44.9	55.9	33.6		62.1	64.2	37.2			53.7
т. н. No.	1940	41.7		31.2	48.9	48.1	38.8	77.3	20.3	23.1	44.3	8.6
3929	1941	26.5	40.1	10.4	21.2	43.6	60.1	86.6	18.2	48.2	40.1	66.4
	1942	55.3	75.2	47.8	40.4	72.4	74.6	78.3	32.7	63.5	59.1	68.4
	1943	22.0	60.0	41.0							47.0	49.8
	Av.	36.4	50.1	36.3	36.0	54.7	61.4	76.6	27.1	44.9	47.8	49.8

^a Averages of 4 replicated plots.

^b Number and place of smut collection: 1a, Nanking, Kiangsu; 2a, Wukung, Shensi; 3a, Tinghsien, Hopeh; 4a, Yaotien, Kansu; 5a, b Number and place of smut collection: 1a, Nanking, Kiangsu; 2a, Wukung, Shensi; 3a, Tinghsien, Hopeh; 4a, Yaotien, Kansu; 5a, Kaifeng, Honan; 1, Kunming, Yunnan; 2 and 3, Loyang, Honan; 4, Kweiyang, Kweichow; 5, Chengtu, Szechwan; 6, Huishiu, Kweichow; 7, Peiping, Hopeh; 8, 9, and 10, Sunghsien, Honan; 11, Malung, Yunnan; 12, Chan-I, Yunnan; 13, Linju, Honan; 14, Tenghsien, Honan; 7, Peiping, Hopeh; 8, 9, and 10, Sunghsien, Honan; 11, Malung, Yunnan; 12, Chan-I, Yunnan; 13, Linju, Honan; 14, Tenghsien, Honan; 15, Lushih, Honan; 16, Huahsien, Honan; 17, Tsinan, Shangtung; 18, Suining, Szechwan; 19, Ping-I, Yunnan; 20, Chengkung, Yunnan; and 21 Luilang, Yunnan. (1a to 5a inclusive were identified in 1936 (3).)

oculate 14 varieties of wheat, including the 4 differential hosts given in table 1, and a variety of poulard wheat known as T. H. No. 559. For comparison, this poulard wheat was also inoculated with the foregoing 11 physiologic races. In the spring of 1943, not a single culm of the poulard wheat, inoculated with these physiologic races, had become smutted. On the other hand, 6.1 per cent of the culms of the poulard wheat, inoculated with the smut from that host, had become smutted. This new smut collection produced different reactions on the common wheats, but the 4 differential hosts, given in table 1, are resistant to it. This additional collection is a distinct race of *Urocystis tritici*. It is, therefore, designated as race 12.

Thus, at the present time, 12 pathogenically distinct races of *Urocystis* tritici have been identified. The varietal reactions that serve to differentiate these races are presented in table 2.

TABLE 2.—Relative susceptibility^a of 5 differential hosts to 12 physiologic races of Urocystis tritici

Differential				. 1	Physi	ologic	race	No.				
\mathbf{host}	1	2	3	4	5	6	7	8	9	10	11	12
Common wheat:												
T. H. No. 1932	R	\mathbf{s}	\mathbf{R}	\mathbf{R}	Ι	\mathbf{R}	\mathbf{R}	Ι	\mathbf{R}	\mathbf{R}	I	\mathbf{R}
N. 716	R	${f R}$	\mathbf{R}	1	I	\mathbf{R}	\mathbf{R}	${f R}$	${f R}$	\mathbf{R}	\mathbf{R}	\mathbf{R}
Ngochen	I	\mathbf{R}	Ι	Ι	Ι	${f R}$	S	Ι	\mathbf{R}	I	\mathbf{R}	${f R}$
Grassland		\mathbf{R}	1	Ι	I	\mathbf{R}	${f R}$	${f R}$	1	S	\mathbf{s}	\mathbf{R}
Poulard wheat:												
T. H. No. 559	R	R	\mathbf{R}	$^{\rm R}$	\mathbf{R}	\cdot \mathbf{R}	\mathbf{R}	\mathbf{R}	\mathbf{R}	R	\mathbf{R}	I

 $^{^{}a}$ R=0-5 per cent infection; I=5.1-20 per cent infection; S=21.1-100 per cent infection.

For aid in identifying the 12 physiologic races of flag smut, the varieties of wheat used to differentiate them are arranged in the following key:

Key for identification of 12 physiologic races of Urocystis tritici

Tsing Hua No. 559 (T. turgidum) resistant Nanking No. 716 (T. vulgare) resistant			F	Physiolo race	gic
Grassland (T. vulgare) resistant Tsing Hua No. 1932 (T. vulgare) resistant Ngochen (T. vulgare) resistant					e
Ngochen intermediate	***************************************		 		1
Ngochen intermediate Ngochen susceptible Tsing Hua No. 1932 intermediate Tsing Hua No. 1932 susceptible	**************		 		7 8
Tsing Hua No. 1932 susceptibleGrassland intermediate	•••••		 *************		2
Ngochen resistant			 		9
Grassland susceptible	*************	••••••			3
Tsing Hua No. 1932 resistant Tsing Hua No. 1932 intermediate			 		10
Nanking No. 716 intermediate					11
Tsing Hua No. 1932 resistant Tsing Hua No. 1932 intermediate					4 5
Tsing Hua No. 559 intermediate	***************************************		 		12

DISCUSSION

The amount of smut produced by a wheat plant depends largely on the amount of moisture in the soil and the temperature during the period of germination (1). The time of sowing, therefore, exerts a great influence upon the strength of attack. In a test of physiologic specialization in *Urocystis tritici*, it is important to bear in mind that the optimum conditions for smut infection should prevail. At Kunming, they occur at the optimum time for wheat planting or a little earlier, about October 15 to 20 when the soil temperatures range from 13° to 25° C. Planting in the latter part of November usually results in a low percentage of smut infection. At that time, the low soil moisture content also becomes a limiting factor.

In the previous investigation (4), the identification of races 1, 2, 3, 4, and 5 was based upon the reactions of Nanking No. 716. This variety of wheat was reported resistant to races 1, 2, and 3, but susceptible to races 4 and 5. In the present studies, it is reported intermediate in its reactions to races 4 and 5 and resistant to all the others. In general, the present results confirm those obtained in the previous studies. Since these 5 races of *Urocystis tritici* have been under observation for 11 years, they seem to be stable in range of pathogenicity.

It is well known that environmental factors may affect the response of host varieties to the causal fungus. Rodenhiser and Holton (2), in their bunt experiments at seven experiment stations, have demonstrated that differences in environment may affect the response of some spring wheat varieties to races of Tilletia tritici and T. levis. In the case of flag smut fungus, the same fact has been observed. For example, H. 1102, a wheat susceptible to races 1 to 5, inclusive, in Nanking (4), was resistant when grown at Kunming. Since the climatic conditions during the growing period of wheat in these two places are so greatly different, it is believed that the host rather than the races show the primary effect of the environment.

As the smut collections used in the present studies were from widely scattered points in China and were so limited in number, it is not yet possible to give the detailed distribution of these races in China. However, 4 of the 7 collections of the smut made in Yunnan have been identified as race 2. Evidently, this race occurs more frequently than the others in this province.

SUMMARY

Twelve physiologic races of *Urocystis tritici* have been separated on the basis of differences in their pathogenicity on 4 varieties of common and 1 variety of poulard wheats. They have been assigned race numbers 1 to 12. Races 1 to 5, previously identified, still remain constant. Races 6 to 12 are described as new.

Races 4 and 5 produce intermediate reactions on Nanking No. 716, a well-known flag smut resistant wheat in China. Race 12, collected on a poulard wheat, is the only race that infects a turgidum wheat.

In Yunnan, 5 races have been identified. Race 2 is conspicuously prevalent and widespread. It occurred 4 times in 7 collections.

Races 1 to 5 have proved stable in pathogenicity for 11 years.

INSTITUTE OF AGRICULTURAL RESEARCH,

NATIONAL TSING HUA UNIVERSITY, KUNMING, CHINA.

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ELSINOË AND SPHACELOMA DISEASES IN YUNNAN, CHINA, PARTICULARLY HYACINTH BEAN SCAB AND SCAB OF CASTOR BEAN

C. C. CHEO AND ANNA E. JENKINS 1 (Accepted for publication January 15, 1945)

Diseases of plants caused by Elsinoë (9) and Sphaceloma (9) have not been noted heretofore in Yunnan, China; it was expected, however, that investigations would reveal their presence. The five diseases of the group here reported from that Province were found in 1938-1939 by the senior writer or by his colleagues of the Tsing Hua University, following removal from Peiping to Kunming. The first three diseases, previously reported from China and known elsewhere in the world, are rose anthracnose, caused by S. rosarum (Pass.) Jenkins (8), grape anthracoose, caused by E. ampelina Shear (17), and sour orange scab, caused by E. fawcetti Bitanc. and Jenkins (2). Previous records of these diseases in China are assembled: no others have been reported from the country. The other two diseases, hvacinth bean scab and castor bean scab, are essentially new; specimens of both from elsewhere in the Eastern Hemisphere were available to the junior writer when the present cooperative study was undertaken. From its nature the present paper should serve as a background for further work on this group of diseases in China. The precedent of referring to citrus scab in China as sour orange scab is followed, although suitable specimens have not yet been available for comparative determination.

ROSE ANTHRACNOSE

Garden roses at the Cheng-ying Primary School at Shi-ping (Szebing),² Yunnan, were severely spotted with rose anthracnose. In China, this disease has previously been reported on wild rose from western Szechwan, north of Yunnan, and from Korea (8).

GRAPE ANTHRACNOSE

In Yunnan viticulture is limited practically to vines planted as a border surrounding the vegetable garden. In August, 1939, leaves and stems of such plants at Pu-Chao, Kai-Yuen, were severely attacked by anthracnose.

In China, outside Yunnan, this disease has been found in widely separated places. Three available records from Nanking consist of a specimen of affected grapes collected in April, 1926, by R. H. Porter (USM 74775),³

¹ This paper was presented in greater detail by the senior writer at the celebration on April 18, 1940, at Kunming, Yunnan, of the 30th anniversary of the National Tsing Hua University. Contributions were to have been published in a special number of "Science Reports" of the University commemorating the occasion. Because of war this plan has not materialized.

² In this paper place names in parenthesis are those of the Chinese official language

(Khan hua) and were furnished by W. T. Swingle.

3 In this paper the symbol "USM" followed by an accession number refers to specimens in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland.

a literature record of the disease also on berries by Teng (20), and a separate account by Chiao (5). Although not previously reported, in 1937 the senior writer noticed the disease on vines in Shangtung Province. He had earlier reported it as causing serious damage in Hopei Province (4). At Changli in this province "long white grape," the most susceptible variety, showed up to 50 per cent infection. At Peiping the local varieties Rose Fragnant and Confort were attacked, Rose Fragnant so severely so that growth was checked prematurely. Among local varieties differences in susceptibility were apparent.

SOUR ORANGE SCAB

At I-liang, Yunnan, sour orange scab was found on fruit of what was apparently mandarin orange by T. F. Yu, T. H. Wang, and S. T. Chao, who contributed representative specimens. At Nan Chi leaves and young fruit of what was possibly a lemon hybrid also was affected. An isolation of the scab pathogen was made and this is referred to in the section entitled "Parallel cultural comparisons."

Sour orange scab is probably old in China, although early records are not directly available. In Japan, where the disease is believed to have been present "from ancient times," original introduction from China is suggested (cf. 19). The earliest available specimen from China consists of diseased rind of "Otehetite orange" from a market in Hong Kong, May 15, 1915, W. T. Swingle (USM 73246). In a recent conversation with Swingle, he stated that this "orange" is probably a hybrid lemon. Mention has already been made (11, Table 1) of scab on the herbarium specimen of Citrus aurantium? from New Territory, China, 1928, C. C. Chum 624, in the Arnold Arboretum. Published Chinese records of scab as assembled by Tai (18) are all from South China, viz., Reinking (15, 16) and Tu (21), To these should be added Lu and Cheo's (12)4 research paper, which reports the first cultural work with Elsinoë fawcetti in China. An observation by them not recorded in their paper is given here as follows: Eureka lemon growing in the citrus plantation at Lingnan Agricultural College, Canton, China, where the investigation was conducted, remained unaffected by scab, although growing among such highly susceptible scabbed varieties as Honglemon and Pei-lemon. Tu's statement relative to the kinds of citrus affected in South China is: "Scab . . . is coextensive with the growing of the Chinese lemon (Citrus limonia Osbeck). The young leaves, twigs and fruits are usually attacked. All the varieties are equally susceptible. All the citrange varieties . . . are extremely susceptible when young. Both Sweet orange and Mandarin orange are either only slightly affected or remain highly resistant under field conditions."

While in Indo-China in 1939, the senior writer noticed scab on Mandarin orange (?) growing in orchards at Lao-kai, Province of Tungkin. The disease appears not to have been reported heretofore from Indo-China, although

⁴ The junior writer is indebted to Dr. Swingle for an English translation of this article from the original Chinese, made in March, 1939, by E. J. Hagerty, University of California.

two specimens are available. The first consists of leaves of Mandarin orange (?) collected on May 19, 1920, by Reinking (USM 69196), the other of citrus rind preserved by R. Kent Beattie from fruit served him at table during his stay at Tourane, Province of Annam, in 1930 (USM 73247).

SCAB OF HYACINTH BEAN

In August, 1938, more than 15 per cent of pods of hyacinth bean (*Dolichos lablab* L.) in a market in Tali, western Yunnan, were affected by the disease here termed "scab of hyacinth bean." Visiting the fields at Er-kai (Erh-hai) where the crop was grown, it was found that all aerial parts of the plant were attacked, severely affected young pods and tendrils having failed to develop or having been killed outright. This same disease was observed also in market in Kunming, both in 1938 and 1939.

It seems probable that this scab of hyacinth bean in Yunnan is the same as that discovered in Kenya Colony (1930) and in Uganda, Africa, a decade or so ago. The finding in Kenya was by the local mycologist E. J. Edwards, who sent a specimen to E. J. Butler, then Director of the Imperial Mycological Institute. Upon finding asci entirely typical of Elsinoë on this material Dr. Butler communicated with the junior writer, to whom part of the specimen was later sent by Dr. S. F. Ashby (USM 72652) in 1936. Hansford's (6) reports of the disease in Uganda are here quoted for convenience of reference as follows:

"The cover crop of *Dolichos lablab* suffered severely from attack by a fungus at present unidentified, but probably related to *Sphaceloma citri* causing the scab disease of citrus. Many of the plants were killed off completely and most showed severe damage to foliage and aerial parts. Affected plants produced very little seed."

"The disease of *Dolichos lablab* reported in 1932 as due to *Sphaceloma* sp., again occured at Serere. Plots of *Canavalia ensiformis* also showed infection. Other legumes were not affected."

A specimen of the Elsinoë on Canavalia from Serere was sent to Dr. Butler, and later divided with the junior writer (USM 72563). The label gives the host as C. ensiformis (?). Dr. Butler's identification of this specimen as E. canavaliae Rac. (14), which is known only on C. gladiata (Jacq.) DC., i.e., sword or saber bean, is probably correct. In view of the fact that the sword bean and jack bean (C. ensiformis (L.) DC.) have been greatly confused as shown by Piper (13, also see 7, p. 1), it seems probable that the diseased Canavalia at Serere was sword bean rather than jack bean. Cross inoculations on both these legumes with the pathogen of hyacinth bean scab made at Yunnan will be reported later. In Yunnan the senior writer has not found Canavalia planted in fields with hyacinth bean; however, in one instance at the Tropical Agricultural Experiment Station at Ho-kow (Ho-K'ou) he observed sword bean growing as a shade plant over hyacinth bean seedlings. The identification of the Canavalia as sword bean was based on

⁵ The fungus referred to here is Elsinoë fawcetti.

Piper's classification (13), *i.e.*, pods about four times as long as broad, 20–25 cm. long, with white seeds.

Symptoms

As observed in Yunnan, the leaf spot is more conspicuous on the lower leaf surface; on the Edwards specimen from Kenya, however, the situation is reversed. On the dry specimens in both instances spots are often "light

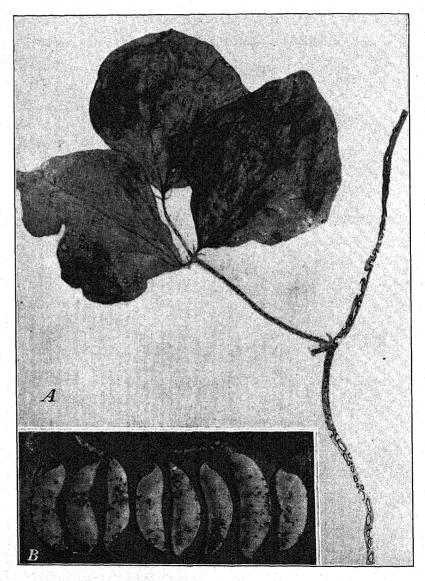


Fig. 1. Scab on lower surface (A) and on pods (B) of hyacinth bean from Yunnan. A, approximately natural size; B, reduced. Photographs by Cheo (A) and C. N. Cheng (B).

huff."6 sometimes faintly bordered with "burnt sienna." and they are often raised at the margin. In their distribution over the blade surface they tend to follow the venation, often involving also the adjacent nonvascular tissue near the midrib or lesser veins. The midrib from the base of the blade practically to the apex may be affected, and the entire apical area also attacked. Where several veins in the same part of the leaf are involved, there may be a general yellowing of the leaf in that region. Interveinal lesions occurring separately tend to be circular, although at the junction of veins, they may be irregularly star-shaped. They may reach 4 mm. in diameter. Symptoms on petioles are similar to those on stems (Fig. 1, A).

On the stems the cankers are circular to linear. They may be numerous and scattered, grouped, or coalescent. Individual lesions range from minute spots to cankers 3 mm. wide by 1 cm. long. They may be flat or slightly depressed, but are often raised, at least at the margin. They are brown at first, becoming ashen, narrowly bordered with pale yellow, or sometimes with dark purplish black (Figs. 1. A. and 2. A).

Pod lesions are more or less circular, punctate to about 5 mm. in diameter. They may be scattered, grouped, or coalescent and where abundant they may produce a mottling effect over practically the entire surface of one or both valves. The suture also may be attacked and lesions here be joined linearly. The spots may be somewhat raised, although not neces-They are brown to purplish brown, becoming lighter at the center, and may be bordered by a band of dark purplish black. The pedicel and calvx as well as the valves may be affected (Figs. 1, B, and 2, A).

The pathogen of hyacinth bean scab (Fig. 2, D, E, F, and Fig. 3) from the sources indicated is here treated as a single species and is described as follows:

Elsinoë dolichi Jenkins, and Bitancourt and Cheo. Ascomata amphigenous, but more abundant on the leaf surface originally infected, on opposite surface produced on or near veins, viewed superficially, appearing as elevations in the dark stromatic rind covering the lesions, also occurring more or less separately as dark punctate bodies, visible as light circular areas where they have caused a rupturing of the rind; in cross section constituting small hyaline to pale yellow pseudoparenchymatic masses often pulvinate and erumpent, with the asci sometimes in one layer although not necessarily so limited, 60 to 300 µ in diameter by as many as 100 u high, often coalescent or at least adjacent; asci subglobose, pyriform to ellipsoid, 20 to 32 µ in vertical diameter by 15 to 22 µ broad, ascospores (immature) 1-septate, probably becoming 3-septate upon maturity, hyaline; imperfect stage consisting of a more or less continuous dark stroma disrupting the epidermis, and developing into the more or less well developed dark stromatic rind, spreading more or less deeply

two new species in Latin were published separately (10).

⁶ Color readings in quotations, are based on R. Ridgway, Color Standards and Color Nomenclature, 43 p., Washington, D. C., 1912.

⁷ Because of the unavoidable delay in publishing this article the diagnoses of the

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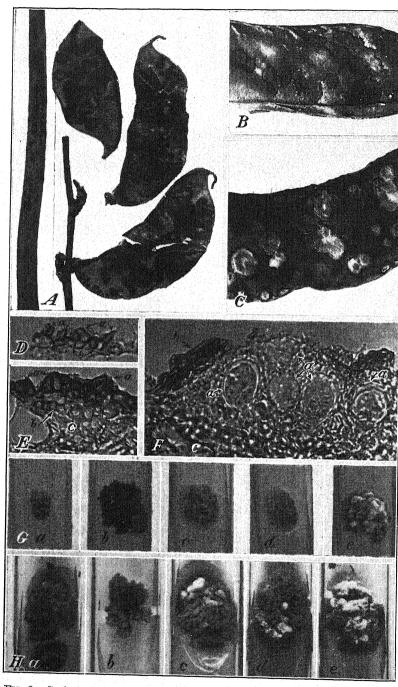


Fig. 2. Scab on pods and stems of hyacinth bean, Kenya Colony, Africa, Sept. 17, 1930. D. C. Edwards. Comm. S. F. Ashby in 1936. B and C. Pod lesions of related legume diseases: (B) scab of *Canavalia* (supposedly sword bean), Los Banos, P. I., Mar. 7, 1920. G. O. Ocfemia (USM 69188); (C) lima bean scab, Palo Seco, P. R., Apr. 12,

into the tissue of the lesions as a hyaline to yellowish prosenchyma, superficially often covered with a well defined dark layer of conidiophores, although for short distances it may be pale, rind including conidiophores attaining 30 μ in thickness, or even in absence of well developed conidiophores sometimes nearly 50 μ thick, composed of small often rounded cells; conidiophores, sometimes arranged in small closely grouped fascicles, consist individually of indefinite to more or less abruptly tapering structures up to 20 μ high and 3.6 to 5.3 μ broad at the base; conidia seen in small numbers only, minute, up to 3.5 μ in diam.; as observed in culture (by Cheo), spherical (2.5–3 μ in diam.) to elliptical (3–7.6 by 1.5–3 μ), hyaline.

Distribution: On leaves, stems, and pods of hyacinth bean (*Dolichos lablab* L.) causing the disease termed "scab of hyacinth bean," Kenya and Uganda, Africa, and Yunnan, China.

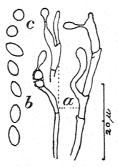


Fig. 3. Elsinoë dolichi. a. Conidial formation from hyphae in a six-day-old potatoagar culture, b and c, conidia. Drawing by Cheo.

Specimens examined: Kenya Colony, Sept. 17, 1930, D. C. Edwards, Type (USM 72652), Phytopathological Herbarium, Instituto Biologico, São Paulo, Brazil (3267), and Imperial Mycological Institute, Kew, Surrey, England; Yunnan, China, Nov. 10, 1938, C. C. Cheo (USM 73032).

Cultures

Elsinoë dolichi was isolated in Yunnan and there grown on potato, malt, and corn-meal agar. The growth was wrinkled and variously colored. On corn meal the main or central part of the culture was covered with livid pink aerial hyphal growth; the agar became violet. The subculture sent to the junior writer was compared with cultures of other species from legumes, namely E. phaseoli Jenkins (3) on lima bean (Phaseolus lunatus macrocarpus Benth.) from Cuba and Sphaceloma arachidis Bitanc. & Jenkins (1)

^{1939,} W. A. McCubbin, Comm. W. A. M. in 1939 (USM 73070). Both A and B, × 1. D–F. Elsinoë dolichi on upper surface of a leaf spot on the Edwards' specimen of hyacinth bean. D. Conidiophores arising from a single hyphal strand (a) or layer (b). E, a, Three clumps of conidiophores; b and c, stroma developed within the disorganized host tissue. F. Ascomycetous stage showing asci (a) imbedded in hyaline stromatic tissue and (b) ruptured epithelium corresponding to the conidial layer, c, host tissue. G. Young cultures, and H, older cultures on potato-dextrose agar; a, E. dolichi; b, E. phaseoli; c, Sphaceloma arachidis; d, S. ricini; e, E. fawcetti. All×1. Photographs (A–H) by M. L. F. Foubert.

on peanut (Arachis hypogea L.) from São Paulo, Brazil. The culture from lima bean was isolated by the junior writer and that from peanut by Bitancourt as shown in the section entitled "Parallel cultural comparisons." The three cultures were distinct.

Inoculations and Cross-inoculations

Hyacinth bean was inoculated with Elsinoë dolichi at Kunming in June and July, 1939. Cultural growth on potato agar was used as inoculum. This was macerated and placed on wet pads of cotton, which were applied to the surface to be inoculated, with the inoculum in contact with the plant. The cotton was kept wet for 48 hours by sprinkling it frequently. At the end of this period it was removed together with the inoculum adhering to it. In the first experiment 15 tender stems, 20 young leaves, lower surface, and 18 young pods were inoculated. In each instance over 50 per cent infection was obtained. Typical scab lesions were distinct on leaves in 9 days and on pods and stems in 12 days. In the second experiment, in which the upper surface of 16 young leaves was inoculated, the results were completely negative. The controls, 15 in the first experiment, 14 in the second, all remained healthy.

In cross-inoculation experiments in Cuba with Elsinoë phaseoli this limabean pathogen failed to infect hyacinth bean, sword bean, jack bean, and several other legumes (3). Sword bean and jack bean are uncommon in Yunnan; seed of sword bean were available from the plants at Ho-kow, and those of jack bean and lima bean were obtained elsewhere. Plants grown from these seed were inoculated with the culture of E. dolichi. The method was the same as that employed for inoculating hyacinth bean, except that both leaf surfaces were inoculated. The results were entirely negative as in the case of the cross-inoculation experiments in Cuba with E. phaseoli. Figure 2, B, represents natural pod infection of Canavalia, presumably sword bean, by E. canavaliae; figure 2, C, that of lima bean by E. phaseoli. The specimens are cited in the legend.

SCAB OF CASTOR BEAN

Castor bean (*Ricinus communis* L.) has long been cultivated for seed in widely scattered localities in southern Yunnan, where it grows as a perennial. Varieties are classified according to petiole color and capsule characteristics.

In June, 1939, the senior writer observed "scab of castor bean" at Gee-kai (Chi-kai) Station and later, in August, saw plants severely attacked at Ta-chong (Ta-Chaung). He had already noticed lesions of the scab on herbarium specimens of castor bean collected at Ta-chong on December 18, 1938, by Yu, Wang, and Chao. In these cases three varieties were affected; Red petiole with small thorny capsule, Red petiole with large thorny capsule, and Green petiole with large thorny capsule. Other varieties that may be affected, as well as actual range of the disease in Yunnan remain to be determined.

Before the disease was discovered in Yunnan it had been found in Formosa by K. Sawada, who sent specimens to the junior writer. These also were severely diseased.

Symptoms

Spots are more prominent on the upper leaf surface and are generally, although not always, visible below. They may be scattered over the blade including the margin; often, however, they are concentrated along the main and lesser veins. At first they appear as small water-soaked areas, which soon turn reddish brown, finally becoming pale, "tilleul buff" and "vinaceous buff," bordered by brown. Where not influenced by veins, individual spots are more or less circular, 2 to 3 mm, in diam. Near veins they may be elliptical to elongate or triangular and on the lower leaf surface in particular, irregularly star-shaped. Vein lesions, below, are inconspicuous, elliptical to linear depressed areas 1.5 mm. wide by 1 cm., more or less, in length. Severe attack along a rib may prevent its normal elongation, with the result that the blade tissue on either side, continuing its growth, becomes wrinkled or waved, and sometimes severed from the diseased rib tissue. The dead papery spots may fall away and the leaf become variously lacerated and torn, as well as blighted where severely attacked (Fig. 4, A-C).

Spots on the petiole and stem are elliptical to elongate, sometimes pointed at each end, reaching 8 mm. in width by 1.5 mm. or more in length. They are at first reddish brown but later become pale, "tilleul buff," at the center, with a brown or purplish black border. This light colored central part may become broken and crumble away. The spots are even with the surface of the petiole or stem, or they may be slightly depressed. They may occur singly, or be grouped and more or less coalescent (Fig. 4, D).

The Pathogen

On the basis of the present study the pathogen of castor bean scab (Fig. 5) is described as follows:

Sphaceloma ricini Jenkins and Cheo. On cankers, conidiophore palisade, yellowish to amber, sometimes practically continuous as a pale dusky layer over all the bordering area, occasionally arched away from the hyperplastic tissue of the canker, with slight or not appreciable pseudoparenchymatous base, 10 to 30 μ , often 25 to 30 μ in thickness, individual clumps of conidiophores sometimes discernible, and where the conidiophores are closely appressed the more slender apices often remaining free from contact with one another, individual conidiophores also sometimes standing apart from one another, observed on leaf spots as well as on cankers, awl-shaped to cylindrical, unbranched, or rarely forked, occasionally abruptly bent, smooth, or apiculate from acropleurogenous production of conidia, apically more or less abruptly pointed, blunt or smooth, where conidia have been produced sometimes spear-shaped, hyaline to pale yellow;

 $^{^8}$ Because of unavoidable delay in publishing this article the diagnoses of the two new species in Latin were published separately (10).

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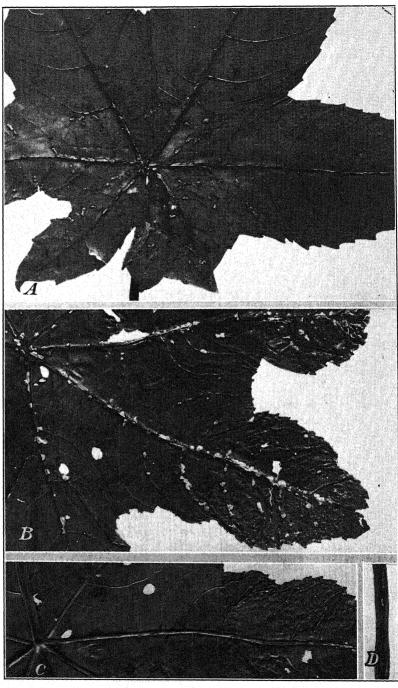


Fig. 4. Leaf spots of castor bean scab. A and B. Upper leaf surface; C. lower surface of part of B. D. Petiole lesions. Specimen from Ta-Chung, Yunnan, C. C. Cheo, Aug. 2, 1939. ×1. Photograph by Foubert.

conidia of various shapes and sizes, oblong to ovoid, elliptical, or fusiform, minute (1–2 μ), up to 10–15 by 2.6–4.5 μ , smaller conidia hyaline, larger and fusiform conidia yellowish.

Distribution: On leaves and stems of castor bean (*Ricinus communis* L.) causing the disease termed "scab of castor bean," Taihoku, Formosa (Taiwan), and several localities in Yunnan, China.

Specimens examined: Taihoku, Formosa, July 2, 1938 (Type, USM 72921) and August 23, 1938 (USM 72934); Ta-chong, Yunnan, China,

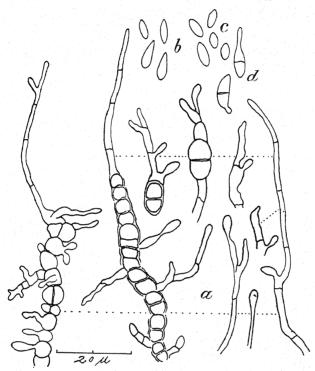


Fig. 5. Sphaceloma ricini. a. Conidial formation from hyphae in six-day-old potatoagar culture, b, c, d, conidia. Drawing by Cheo.

December 16, 1938, T. F. Yu, T. H. Wang, and S. T. Chao (Herb. Nat. Tsing Hua Univ.), Aug. 2, 1939, C. C. Cheo (USM 73162); Gee-kai, Yunnan, June, 1939, C. C. Cheo (Herb. Nat. Tsing Hua Univ.).

Cultures

Sphaceloma ricini was isolated at Kunming and grown on potato, malt, and corn-meal agar. A black sector produced in one instance was isolated and remained constant throughout many transfers. A black sector also developed from the subculture sent the junior writer. The aerial hyphae of the culture on corn meal were white, and the medium became violet, although not the same shade as in the case of the corresponding culture of Elsinoë dolichi. Conidia produced in culture at Yunnan are represented in figure 5.

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Inoculations

The method of inoculating castor bean plants was essentially the same as that employed in the experiments with *Elsinoë dolichi*. In general the upper leaf surface was inoculated, as this is obviously more susceptible to infection than the lower leaf surface.

Infection was obtained on the four varieties inoculated, namely, Green petiole variety with large smooth capsules and that with large thorny capsules, and the Red petiole variety with large thorny capsules and that with small thorny capsules. Good infection was obtained in all cases except in the instances where the inoculum was placed on the lower leaf surface or where the inoculum was the black strain of the fungus. The black strain, originating as a saltation, was a weak pathogen. The time required for the lesions to become visible or distinct ranged from 7 to 15 days with one instance of leaf discoloration in 2 days. The lesions in all cases were entirely typical of those of scab on castor bean observed in nature. Controls for each of the experiments remained healthy.

PARALLEL CULTURAL COMPARISONS

The parallel cultural comparison is represented in figure 2, G, a to e and H, a to e. The first series of these Thaxter's potato agar cultures grew for two weeks at room temperature. In the second series, which is a separate set of transfers, all but H, c, were grown one month at room temperature, then held for two months at a lower temperature (refrigerator) which permitted slight growth. These cultures were thus three months old when photographed. The culture in H, c, represents six weeks' growth at room temperature.

Elsinoë dolichi, E. phaseoli, and Sphaceloma arachidis, in the order named, are represented in figure 2, G, a-c, and H, a-c. The two-week-old cultures (Fig. 2, G, a-c) were of the following colorations: E. dolichi, "clay color," E. phaseoli, "carbon brown," S. arachidis, reading from the outside to the center, "cinnamon buff," "avellaneous" and "buffy brown." Throughout their development the cultures in figure 2, H, a-c, remained distinct from each other; that of E. dolichi became chromogenic, "sorghum brown." At the age of 8 months the thallus of E. dolichi had become "Victoria lake" to nearly black; that of E. phaseoli was "fawn color" at the center and in other parts, "Hessian brown," "diamond brown," and black. Comparable growth of the corresponding culture of S. arachidis was generally drab, i.e., "vinaceous drab," "light Quaker drab," and "Quaker drab."

⁹ Thaxter's potato agar was made by Miss Agnes Quirk of the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, as follows: 500 g. potato (mature and sound), 1,000 cc. distilled water, 20 g. dextrose, 15 g. agar. Slice potatoes thin, immediately add distilled water, steam 20 min. in the Arnold sterilizer, or let simmer in a water bath, temperature below 60° C., for one hour. Filter through cheesecloth, make up to original amount with water. Add 2 per cent dextrose and 1½ per cent agar and cook for one hour; filter through cotton, tube, and autoclave tubes 20 min. at 115° C.

Figure 2, G, d, and H, d, represents the culture of Sphaceloma ricini. At the end of the first month the culture in H was "ochraceous brown" in the outer part and "Mars brown" at the center, with the medium chromogenic, "lime green" and "mignonette green"; after 8 months, thallus "cinnamon" with a mixture of other colors, medium uniformly "mignonette green."

The culture of Elsinoë fawcetti in the two series (Fig. 2, G, e, and H, e) was of essentially the same coloration; at the end of one month "ochraceous buff" at the outside and "walnut brown" deepening to "maroon" at the center; aged 8 months, "ochraceous tawny" in the outer part, "pale vinaceous drab" and "light vinaceous drab" over a large area at the center, where there were aerial hyphae.

SUMMARY

Five diseases caused by Elsinoë and Sphaceloma were discovered by the senior writer in Yunnan Province in 1938–1939. These are rose anthracnose, grape anthracnose, and sour orange scab, of which previous records elsewhere in China are assembled. The two other diseases, hyacinth bean scab and scab of castor bean, are essentially new. Without description, the presence of hyacinth bean scab in Uganda, Africa, had been reported by Hansford (1932-33); also part of the D. C. Edwards' specimen from Kenya Colony, 1930, had been made available to the junior writer (1936). Similarly, Sawada had sent specimens of castor bean scab from Formosa prior to the receipt of Cheo's specimens from Yunnan. Symptoms of these two new diseases are delineated and their pathogens described, the organism from hyacinth bean as Elsinoë dolichi Jenkins, Bitanc. and Cheo, that from castor bean as Sphaceloma ricini Jenkins and Cheo. (Diagnoses in Latin were made available in 1941.) Inoculations with cultures of $S.\ ricini$ on four different castor bean varieties gave positive results, as did corresponding experiments on hyacinth bean with E. dolichi. Only negative results were obtained in cross inoculations with E. dolichi on sword bean, jack bean, and lima bean. Parallel cultural comparisons on Thaxter's potato agar included one culture each of E. dolichi, two other legume species, E. phaseoli on lima bean from Cuba and S. arachidis on peanut from São Paulo, Brazil, Sphaceloma ricini and E. Lancetti. The cultures were distinct in all instances.

TSING HUA UNIVERSITY, KUNMING, YUNNAN, CHINA, AND BUREAU OF PLANT INDUSTRY STATION, SOILS AND

AGRICULTURAL ENGINEERING,

U. S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND.

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THE FIELD INOCULATION OF RYE WITH CLAVICEPS PURPUREA¹

RALPH W. LEWIS

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INTRODUCTION

This paper presents a new technique for the inoculation of rye with Claviceps purpurea (Fr.) Tul. The method consists primarily of producing an artificial honeydew by mixing saprophytically grown spores with a strong sugar solution. In this solution the spores can be stored for months without great loss of viability. Spores do not die when drops of the sugar-spore-suspension dry out, and will germinate readily when water is added. In these respects the suspension resembles natural honeydew. A special sprayer was devised to apply the diluted spore suspension to the blooming rye plants. An abstract of this paper was previously presented to Phytopathology (9).

The results of this work may be applied to commercial production of ergot and to many scientific problems such as the relation of insects to ergot, the testing of grasses bred for resistance to ergot, the taxonomy of species of *Claviceps*, and the study of physiologic specialization within a species. An artificial matrix suspension might be used for other fungus or bacterial plant pathogens whose reproductive cells are naturally produced in a matrix.

REVIEW OF THE LITERATURE DEALING WITH THE LARGE-SCALE INOCULATION OF RYE WITH ERGOT

Fairly successful methods for the large-scale inoculation of rye with ergot have been worked out by Bekesy (3) and Hecke (5, 6). Bekesy's method is based on the use of a horse-drawn, multiple injecting apparatus which deposits spores inside the closed flowers, while Hecke's method depends upon a considerable amount of hand manipulation to cause the flowers to open and to apply the spores. Hecke's method could not be used for commercial production of ergot in the United States because it requires too much hand labor. Bekesy's apparatus might be adapted to commercial production provided the machine does not cause too much injury to the rye, provided it will stand the wear and tear of much use, and provided most of the infections arise from the initial inoculations. Any method depending upon natural spread is not reliable enough for the propagation of ergot in most locations where rye is grown.

McCrea (10), Hynes (8), and Thomas and Ramakrishnan (11) have tried large-scale inoculations by spraying aqueous spore suspensions on the rye when the plants were in bloom. McCrea's results using a horse drawn

¹ Condensation of a thesis presented to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the Degree of Doctor of Philosophy. Approved by the Director of the Michigan Agricultural Experiment Station as Journal Article No. 721 (n.s.).

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sprayer were so inconclusive that she says in her summary, "Field demonstrations have shown it to be improbable that parasitic culture of C. purpurea on a large scale would be desirable, under prevailing conditions in Southern Michigan." Hynes says of the results secured by application of an aqueous spore suspension by various means on 200 acres of rye: "Dry seasonal conditions generally were adverse to ergot formation. Consequently, yields were low (½ lb. to 20 lb. per acre), but ergots did develop in most instances—even in the driest areas sown to rye." Thomas and Ramakrishnan sprayed their plots twice and a few infections occurred after the first treatment. Ergot subsequently spread to the whole field, even to the untreated plots. Their results were better than those of McCrea and Hynes, but most of their sclerotia apparently arose from secondary infections, hence a good yield would be dependent upon favorable weather.

Many investigators have had success in small plots by spraying on an aqueous suspension of spores. The results of Fron (4) and Hynes (8) illustrate this. Heckt (7) was granted German and U. S. patents on a hand-operated, multiple injecting apparatus for inoculating rye through the palets and lemmas of closed flowers. He claims that large-scale inoculations can be made, but here, again, much hand labor would be necessary. Barger (2) and Bekesy (3) give good reviews of the literature dealing with attempts at large-scale inoculations with ergot. They also discuss an important phase of the problem not touched upon in this work: the use of more susceptible varieties of rye in order to get maximum infection with minimum inoculation effort.

SEARCH FOR A SPORE MATRIX AND STORAGE OF SPORE SUSPENSIONS

Observations in the field and information gained from the literature gave rise to the hypothesis that the most effective agent for inoculating rye with Claviceps purpurea would be a spore suspension with properties similar to the sphacelial suspension produced in nature. To be similar, an artificial suspension must: (1) prevent immediate germination, (2) protect the spores from death by desiccation after application, (3) attract insects, (4) allow germination once the spores come in contact with the pistils of the rye flowers.

Maple sirup, light and dark corn sirups, honey, and a concentrated solution of beet sugar were tested to determine their effect on the vitality of spores suspended in them. Only the beet sugar gave promising results. Table 1 gives the percentage germination of samples removed from quart bottles of 50 per cent beet-sugar-spore-suspension. Spores in a sugar solution matrix will remain viable for months at a low temperature. In tests where germination decreased considerably, large numbers of bacteria were usually present and the decrease was attributed to them.

The percentage germination was determined at first by estimation and later by a smear method. A drop of suspension was placed on a grease-free, sterile slide, smeared to form a thin film, and the slide placed on wet paper in a Petri dish. After incubation for about 18 hours at 28° C., the slide was

TABLE 1.—Percentage germination of samples from sugar-spore-suspensions stored at various temperatures

Days suspension in storage before-		Storage ten	nperatures, d	legrees C.	
samples taken	-18	0	12–16	20-26	30
3	60	80	60	70	60
18	40	60	50	0	0
38a	70	70	0		*****
42	40	40			
61	40	43		*****	
128	45	19			

a Percentages estimated on samples up to 42nd day; others determined by actual count.

allowed to dry. A cover slip with a small drop of water was placed on the slide. Ten high-power fields were counted, combined, and the percentage of germination computed. From time to time the sugar-spore-suspensions in storage were examined and no germination occurred, not even in those stored at room temperature.

In sugar concentrations from 34 to 66 per cent, spores remained viable even when drops of the suspensions were allowed to dry in room air or over calcium chloride for 5 days. Neither the variation in sugar concentration nor the drying of the suspension reduced the percentage germination. This is important because it means that spores applied in the field will remain viable for a number of days even though the weather is dry.

PREPARATION OF SPORE SUSPENSION

Cultures for the mass production of conidia were grown in quart milk bottles using the method of Hynes (8): 250 ml. of wheat and 250 ml. of water were mixed in a bottle, allowed to stand overnight, and autoclaved one hour at 15 lbs. A piece of a heavily sporulating stock culture was added and mixed thoroughly with the wheat medium by pounding on a rubber stopper. All cultures were grown at room temperature and harvested at the end of five to six weeks. The cultures were mixed with an equal volume of tap water and beaten in a blender, a method of preparing inoculum described by Andrus (1). After blending for about two minutes the suspension was screened through a 16-mesh and then a 40-mesh screen. To this thick, water suspension of spores, medium, and mycelium an equal weight of beet sugar was added and stirred until dissolved. Five gallon honey cans were used for storage at -18° C. and 0° C. About $4\frac{1}{2}$ gal. of the sugar-spore-suspension was made from 10 quarts of culture.

FIELD INOCULATIONS

A field trial in 1941 using traps for catching flies, exposing them to a culture of *Claviceps purpurea* and releasing them, failed completely to bring about any infections, but did give rise to the basic hypothesis of this work. The first trials using a sugar-spore-suspension for inoculation were made in

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1942 and on four plots an average of 9 per cent of the heads were infected as compared to 1 per cent on the control plots. In 1943, five fields were inoculated, three with a power sprayer and two with a hand sprayer. Two of these fields are described here, with only a few statements about the others.

The Clark Field

This field of Rosen rye contained six blocks in a row. Each block was 80 ft. long and made up of five lengthwise plots 6 ft. wide arranged with roadways to make them accessible to a sprayer. The spraying was done with a small orchard sprayer (Fig. 1). The boom carried besides the nozzles three

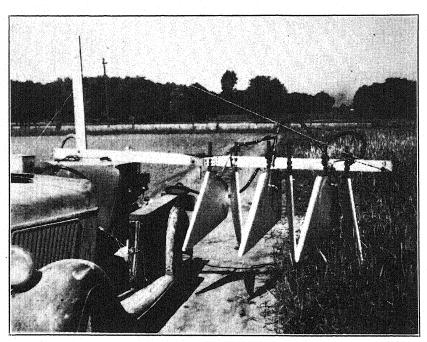


Fig. 1. The power sprayer used to apply the sugar-spore-suspension to the rye flowers.

pairs of guides whose construction and arrangement can be seen in the picture. At the time this field was sprayed there was only one nozzle between each pair of guides and it was adjusted to point into the tops of the rye heads as they passed between the guides.

*Information concerning the number of treatments and strength and quality of spore suspension is in table 2. The plots were sprayed once each day on June 8, 9, and 10. Plots in blocks I and V were sprayed once more on June 11. Spraying was done in the morning except on June 9 when the field was treated in the early afternoon. Duplicate plots in blocks I, II, and IV were combined and in blocks V and VI all plots were treated alike. The middle plot in each block was untreated.

In every block, regardless of the kind of treatment, there was a great

difference between the sprayed and unsprayed plots. The lower quality suspension, with 10 to 30 per cent germination, was less efficient in producing infections than was the better quality one. The highest dilution of suspension used was just as effective as the more concentrated suspensions. This substantiates similar results from another experiment. The control plot in all treated blocks was in immediate contact with one treated plot and separated from the next by a 6-ft. roadway. Carry-over was small, but probably accounts for a part of the infection in the controls. The higher percentage of ergot in block III as compared to the controls in the treated blocks was probably due to its location in a lower part of the field. With the commer-

TABLE 2.—Treatments and results on Clark Field

Block No.	Number of treatments ^a	Plot	Strength of suspension	Percentage of heads infected ^b
I	4	A B C	1:1 1:3 (untreated)	43 38 5
II	3	Ă B	$egin{array}{c} 1:1 \ 1:3 \end{array}$	36 30
III IV	0	C A	(untreated) 1: 7	3 8 33
\mathbf{v}	4	B C A & B	$\begin{array}{c} 1:15\\ (\text{untreated})\\ 1:1 \end{array}$	35 3 25
VI	3	C A & B	(untreated) 1:1	$\begin{matrix} 25 \\ 6 \\ 22 \end{matrix}$

<sup>Blocks I, II, and IV were treated with spore suspensions whose germination was 40-60 per cent; blocks V and VI with suspensions of 10-30 per cent.
An average for the whole field of 1.8 sclerotia per infected head.</sup>

cial production of ergot in mind, it is noteworthy that the treated area was more than one quarter of an acre and that a rapidly moving sprayer applying a sugar-spore-suspension inoculum caused a fairly high percentage of infection. This was the first attempt to apply this type of suspension with a power sprayer and the whole operation was crude when considered in the light of the experience gained.

Flies, present in large numbers on the 1942 plots, were almost absent on the Clark Field. Honey bees, however, were plentiful during the treating period, especially on those plots with the highest concentrations of suspension. Ergot beetles (Acylomus ergoti Casey) were first seen in block I on June 20 and continued to be present throughout the field while the sclerotia developed. At no time were they as plentiful as on the Soils Field where there was an average of one beetle per head. From the few observations made, no relationship between the insects and the number of infections was established. It is probably true that insects are not a very important factor in the spread of ergot if the rye blooms and ripens evenly over the whole field.

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During the time that this field was being treated the weather was bright and warm with only a trace of rain on the first day and with average humidity. From the results of this experiment and observations of others, it is believed that the success of this method of inoculating rye is not dependent upon hot, humid weather which is usually associated with natural epiphytotics of ergot. Because rain washes the suspension off the heads, it is detrimental. If the droplets remain from day to day, there is a good chance that as the flowers bloom the stigmas will come in contact with the droplets of suspension on the outside of the heads.

The Soils Field

Two adjacent plots of Rosen rye, 15×25 ft., were planted in October, 1942. Next to these, two plots of spring rye, each 30×25 ft., were planted in April, 1943. One of the fall rye plots was sprayed, using a hand sprayer, with a 1:1 dilution of high quality spore suspension, at the rate of 150 gal. per acre, between 8 and 10 a.m. on June 12, 14, 15, 16, and 17. This was during the period of most rapid blooming. The spring rye plots, which were untreated, began to flower June 22 and continued to bloom for 6 days during the maximum honeydew formation on the fall rye.

Sixty-one per cent of the heads in the treated plot, 10 per cent in the untreated fall rye plot, and 28 per cent in the spring rye plots produced sclerotia. The strip of spring rye 6 ft. wide adjacent to the treated plot had 44 per cent of the heads infected while in the most distant part there were only 23 per cent. For the whole field there was an average of 2.0 sclerotia per infected head.

Other 1943 Fields

One field sprayed three times with a hand sprayer using different concentrations of suspension produced rather poor results. The treated plots ranged from 10 to 20 per cent of the heads infected with less than 1 per cent in the controls. These poor results were probably due to the cool, misty weather that prevailed during half of the blooming period rather than to the method of application. Under these conditions the suspension of spores was washed off and the flowers did not open normally.

Fairly good results were obtained from one field which was treated three times with the power sprayer between 10 and 12 a.m. one day when the rye was at the peak of its bloom. Thirty-four per cent of the treated heads became infected, with 5 per cent in the controls. A field of spring rye treated with the power sprayer gave rather poor results when the total number of applications is considered. Three applications each day on three separate days with a 1:7 dilution caused only 60 per cent infection. The controls had 13 per cent of the heads infected. The poor results were probably due to the fact that spring rye grows poorly in Michigan: the plants were small and the heads very poorly filled. Perhaps a large number of flowers were infected without subsequently producing sclerotia.

GENERAL DISCUSSION

By weighing a number of medium sized sclerotia it was calculated that there are approximately 5,000 sclerotia per pound. On the basis of 1,200,000 heads of rye per acre the approximate yield of ergot in lbs. per acre can be computed by multiplying 1,200,000 by the percentage of heads infected, multiplying by the average number of ergots per head and dividing by 5,000. The figures thus obtained are likely to be misleading from a commercial point of view because of losses in harvesting. It was felt that acre yield estimates would be unreliable until later work with larger plots would actually show that a certain number of pounds of ergot could be harvested from large areas.

The problem of weather in relation to this method of inoculating rye with ergot has not been solved by the experiments presented, nevertheless two questions appear to be partially answered. Rain is definitely detrimental because it washes off the sticky droplets of suspension so they fail to come in contact with the pistils of later opening flowers. This was observed in 1942 and in 1943. Bright, clear weather appears to be favorable. Three of the four days during the treatment period on the Clark Field were bright; the first day had a trace of rain, but there was enough sunshine to dry it rapidly. Further research will be necessary to secure definite answers to the many questions about the relationship of weather of this method of inoculating with ergot.

It is obvious from information secured in the Clark Field that ergot does not spread far in a field of rye which blooms evenly during bright weather. The control strips which were adjacent to treated strips had a small percentage of infection, most of which could have been caused by blowing of the spray at the time of treatment. Probably the best evidence of failure to spread was obtained by examining the field of which these plots were a part. Only by careful searching could a sclerotium be found even within ten feet of the treated areas. Many authors have made note of this, but it bears repeating, because it is one of the first questions that comes up when one thinks of producing ergot artificially. It is obvious from the results of the Soils Field that if later blooming rye is nearby there may be considerable spread. This will undoubtedly also be true of grasses that are susceptible to the biologic races of ergot which attack rye.

The best time for the application of the spore suspension in relation to the blooming of the rye remains to be accurately determined. Rye flowers bloom in recurring intervals over a whole field. For 15 minutes a large percentage of the heads have from 1 to 5 flowers open. These close rapidly and for about 45 minutes almost no flowers in the field are open; then the blooming cycle begins again. The cycles begin about the time the sun strikes the field and continue all morning. The number of flowers that open in each cycle diminishes through the early afternoon so that by late afternoon blooming almost ceases. Cloudy weather and temperature changes influence the rhythm of bloom. These observations have not been carefully checked and

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However, based on them, it was concluded that are of a tentative nature. the best time to spray is between 7 and 11 A.M.

A number of factors need further research before it can be said that the full capacity of this method to produce ergot is reached. Some of these are: (1) the most efficient method of applying the spore suspension, (2) the strength of the spore suspension, (3) the strength of the sugar solution used for the suspension, (4) the time of application, (5) the rate of application, (6) the number of applications, (7) the best age of culture to harvest for spores, (8) the best strain of the fungus, and (9) whether or not all applications should be made on one day or on different days. Add to these some factors which are general to this problem—(1) the variety of rye, (2) the site of the field, (3) the geographical location of the field, (4) machinery for harvesting the sclerotia—and one can see, in the light of the results already secured, that the possibilities of producing ergot artificially are good if the optima for these variables are determined.

SUMMARY

A method is described for the preparation of an ergot spore suspension which can be kept in cold storage for weeks and probably months, then diluted and used to inoculate rye plants in the field by spraying on the plants at blooming time. The suspension consisted of beaten and screened cultures to which was added an equal weight of beet sugar. A machine for the application of the spores is described. The results indicate that this is a good and relatively simple method of inoculating rye with ergot.

The writer wishes to express his thanks to Dr. E. A. Bessey for his assistance in carrying out this research.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, MICHIGAN STATE COLLEGE, East Lansing, Michigan.

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THE EFFECT OF BRIEF TEMPERATURE TREATMENTS ON GERMINATION OF UREDIOSPORES OF PHRAGMIDIUM MUCRONATUM (FR.) SCHLECHT.1

VINCENT W. COCHRANE

(Accepted for publication February 17, 1945)

In the course of studies on the rate of germination of urediospores of Phragmidium mucronatum (Fr.) Schlecht, cause of the common leaf rust of cultivated roses, it was found that germination at low temperatures was greatly increased by exposure of the spores to room temperature during the period needed to make microscopic counts. In view of the fact that this exposure never exceeded 10 minutes, the phenomenon was judged of sufficient interest to warrant further investigation.

MATERIALS AND METHODS

A single-urediospore line of *Phraamidium mucronatum* was cultured on. the Hybrid Tea variety Briarcliff. This clone was obtained from the rose variety Christopher Stone, grown in California.2 Mature urediospores were brushed from infected leaflets into a beaker and transferred to a miniature "puff-duster" made of glass tubing. The spores were blown onto the surface of 5.0 cc. of 2 per cent water agar contained in a Syracuse watch glass. The watch glass and agar were held at the planned temperature of incubation for at least 8 hours before the spores were sown. Sown plates were stacked to prevent drying of the agar, and were incubated at the desired temperature.

Germination was counted microscopically in areas of the agar plate containing 75-150 spores per sq. mm. In each replicate 250 spores were counted for each observation; 2 or 4 replicate plates were sown for each treatment. The significance of differences between treatments and the agreement of replicates were assessed by the Chi-square method.3

Previous experiments had shown 18° C, to be the optimum temperature for urediospore germination, and all check lots were held at this tempera-The limits of urediospore germination are 6° and 28° C.; germination in appreciable percentage occurs only over the range 9-25° C. Temperature chambers used were equipped with thermoregulators accurate to \pm 1° C.

In the first group of experiments spores were sown on agar at a low temperature, 6°, 8°, or 9° C. At stated intervals over a period of 36 hours 2 or

1 Excerpt from a thesis presented April, 1944, to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy.

² The writer is indebted to Dr. H. Earl Thomas, Dept. of Plant Pathology, University of California, Berkeley, Cal., for the original supply of rusted leaves.

³ McCallan, S. E. A. and F. Wilcoxon. The precision of spore germination tests. Boyce Thompson Inst. Contrib. 4: 233–243. 1932.

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4 of the plates were placed for a short period (10 minutes) in an incubator at 27° C., then returned to the original low temperature. Thus, at the end of an experiment there were available for comparison plates which had been warmed at each of several different time periods. Germination on these various plates was then determined and compared with that of spores on plates held uninterruptedly at the low temperature.

TABLE 1.—The effect of a 10-minute exposure to 27° on spores germinating at 6°, 8°, or 9° C. Watch glasses containing germinating spores on agar were taken from the low temperature chamber at various intervals during the germination process and were held 10 minutes at 27° C., then were returned to the low temperature chamber. Germination on the treated plates was counted 24 hours after the time of exposure; germination of the control was counted at 48 hours from the time of sowing

Experiment	Base temperature	Exposed at (hours from sowing)	Total counted	Per cent germination ^a	Per cent increase ^b over control
1	9° C.	(control)	500	68.2	0.0
		4 hr.	500	94.0	37.8**
		8	500	92.4**	35.5**
		12	500	87.2	27.9**
		24	500	79.4	16.4**
		36	500	65.6	-3.8
		48	500	70.6	3.5
2	8° C.	(control)	1000	28.3	0.0
		2 hr.	1000	52.3	84.8**
		4	1000	59.3	109.5**
		6	1000	63.8	125.4**
		8	1000	50.0	76.7**
		12	1000	44.9**	58.7**
		24	1000	33.8	19.4**
		36	1000	30.5	7.8
3	8° C.	(control)	500	22.8	0.0
		2 hr.	500	65.8	188.6**
		4	500	64.6	183.3**
		6	500	65.8	188.6**
		8	500	61.6	170.2**
		12	500	46.4	103.5**
		24	500	17.4	-23.7
		36	500	19.2	-15.8
4	6° C.	(control)	1000	5.7	0.0
		2 hr.	500	29.2	412.3**
		4	500	30.8	440.4**
		6	500	30.0**	526.3**
		8	500	17.6	208.8**
		12	500	9.4	64.9**
		24	1000	5.3	-7.0
		36	500	5.2	-7.0 -8.7

^a Double asterisk in this column indicates significant (99:1) difference among replicates.

b Double asterisk in last column indicates significant (99:1) increase over control.

The data of 4 experiments are in table 1. The time of exposure (Column 3) was measured from the moment of sowing. Thus, for example, the second line in table 1 represents the germination of 2 replicate spore samples held 4 hours at 9°, then 10 minutes at 27°, and finally 24 hours at 9° C. The control in each case was the germination of spores held continuously at the low

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temperature. Total viability was determined by germinating a sample of spores at 18° C., and in no experiment was germination at this temperature lower than 90 per cent.

The figures of the last column of table 1 express the magnitude of the increase in germination caused by the exposure to 27° C. In all cases the effect of the treatment was significant at exposure times up to 12 hours from the time of sowing, and in two experiments (1 and 2) significant increases in germination resulted from exposure at 24 hours. The effect was greatest during the first 8 hours, declining thereafter and disappearing at 36 hours (24 hours in Experiment 3 and 4). In other words, the spores cease being receptive to the stimulus of the warming after 12–24 hours, and the maximum effect of such treatment is obtained when the exposure is within 8 hours of sowing the spores. In this connection, it may be noted that, with spores held continuously at 9° C., germination is first evident 4 hours after sowing, and that no increase in germination occurs beyond 10 hours from sowing of the spores.

When the period of exposure to 27° C. was reduced to 5 minutes, increases of up to 90 per cent were obtained; in this case the base temperature was 9° C.

A population of senescent spores responded in the same way as the fresh spores. Germination at 9° C. was 8.1 per cent in this case; exposure to 27° C. for 10 minutes at 6 hours from sowing raised the germination to 36.4 per cent. A sample from this population proved to be 83.5 per cent germinable at 18° C.

A further experiment, using a base temperature of 3° C. and fresh spores, was performed. In this case exposure to 27° C. for 10 minutes failed to bring about any germination; as noted previously, no germination occurs at 3° C.

One possible explanation of these results is the occurrence of condensation when the cold plates are set in a warm chamber. To examine this possibility, in one experiment sterile tap water was substituted for agar as the germination medium. The results were in all respects similar to those on agar: increases of up to 70 per cent were obtained by a 10-minute exposure to 27° C. of spores germinating at 9° C.

A second possible factor is exposure of the spores to light during the transfer from one chamber to another. To test this, two experiments were made to investigate the effects of warming of plates wrapped in black paper. Although the wrapping in paper reduced germination in all plates, the response to warming was proportionately the same as in unwrapped plates. Thus, in one case exposure of wrapped plates for 10 minutes to 27° C. (incubation temperature 9° C.) 4 hours after sowing increased germination from 8.0 per cent to 31.2 per cent.

No measurements of the temperature at the agar surface were made. "Exposure to 27° C. for 10 minutes" does not, therefore, imply that the treated spores reached or were maintained at this temperature. The Syra-

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cuse watch glasses bearing the spores were not stacked during the exposure to the high temperature, but were spread out singly. The spore on the agar surface was thus presumably separated by only a thin layer of water from air at 27° C. Undoubtedly the spores rose in temperature, but the precise amount of increase was not determined.

A related phenomenon was investigated in the experiment summarized in table 2. Agar plates sown with urediospores were held for one hour at 18° C., then for 23 hours at various temperatures known to be non-optimal for germination (3°, 6°, 9°, 24°, 27°, and 30° C.). No germination occurs during the first hour of incubation at 18° C. After 23 hours at the various temperatures germination of each lot was determined and compared with that of control spore samples incubated the entire 24 hours at the same tem-

TABLE 2.—The effect of a preliminary one-hour exposure of germinating urediospores to 18° C. on subsequent germination at various unfavorable temperatures. Spores were sown on 2 per cent water agar. Treated spores held one hour at 18° C. and 23 hours at an unfavorable temperature were compared with control spores held 24 hours at the unfavorable temperature. Each per cent germination, except as noted, was based on a count of 250 spores in each of 4 replicates

Temperature,	Per cent germination	Per cent increaseb		
°C.	Control Treated	over control		
3	0.0 1.7			
6	0.8 6.6	725.0**		
9	6.5	163.1**		
18	96.6			
24	77.6 90.0a	16.0**		
27	5.0 20.4a	308.0**		
30	0.0			

^a Based on a count of 250 spores in each of 2 replicates.

perature. In table 2 each lot of treated spores is compared with spores held for 24 hours, with no preliminary incubation at 18° C., at the unfavorable temperature.

From the data of table 2, it is evident that the one-hour exposure to 18° C. had a significant positive effect, even though at the end of the one-hour period no germination had been visibly initiated.

Consideration of the data of table 2 suggests a point of some interest in relation to methods of spore germination. In temperature trials of germination it would seem desirable to insure that the germination medium is at the control temperature before the spores are sown, rather than to sow the medium at laboratory temperature and only then to place the vessels at the experimental temperature.

Evidence on this methodological point was obtained and is presented in table 3. A comparison was made between agar plates sown at 18° C. and placed immediately at the low experimental temperature (7° or 9° C.), and similar plates held 24 hours at the low temperature before sowing. The latter are designated "precooled."

b Double asterisk indicates significant (99:1) increase over control.

In temperature studies of spore germination the medium should be brought to the desired temperature before the spores are sown. Some investigators⁴ have taken this precaution, others probably have not.

DISCUSSION AND SUMMARY

Large increases in germination of urediospores of *Phragmidium mucro-natum* at 6–9° C. were brought about by brief warming of the germinating spores in an incubator at 27° C. The increases were greatest when exposure to the high temperature was made during the first 8 hours after sowing of the spores; exposure at periods of more than 24 hours after sowing had no effect.

The actual temperature of the agar during the warming in the high temperature chamber was not determined; it is therefore impossible categori-

TABLE 3.—The effect on urediospore germination at low temperatures of a preliminary cooling of the germination medium to the control temperature. Germination on precooled agar was compared with that on agar sown at room temperature (20° C.) and then placed at the low temperature. Germination at 18° C. was 93.9 per cent. Each per cent germination was based on a count of 250 spores in each of 4 replicates

Temperature	Treatment	Per cent germination ^a
7° C.	Precooled Not precooled	6.1 24.4**
9° C.	Precooled Not precooled	50.1 79.0**

^a No significant differences among replicates. Double asterisk indicates significant (99:1) increase over germination on the corresponding ''precooled'' agar.

cally to state that any particular temperature is decisive. The results suggest that there may exist some reaction—or reactions—in the germination process which is limiting at 6-9° C. and which can be completed in a relatively short time at a higher temperature.

The germination of urediospores at temperatures too high or too low for optimum germination was significantly increased by a preliminary one-hour incubation at 18° C., the optimum temperature for germination in this species. Again, the speculation that a temperature-sensitive key reaction may be completed during the period at 18° C. is suggested by these data.

From the standpoint of spore germination methods, these results indicate that extreme care is necessary, in temperature tests of germination, to insure that the germinating spores are always at the desired temperature. In practice this requires a preliminary incubation of the germination medium at the desired temperature. Further, in studies on the rate of germination, the data show that either counts should be made at the control temperature or the samples counted at a higher temperature should be discarded.

⁴ Straib, W. Physiologische Untersuchungen über *Puccinia glumarum*. Centr. Bakt. II, 102: 154–188. 1940.

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In nature a short period of favorable temperature may result in spore germination on the leaf even though temperature is generally unfavorable. This consideration suggests caution in applying the results of laboratory experiments to field problems.

DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY, ITHACA, NEW YORK.

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PHYTOPATHOLOGICAL NOTES

Effect of Vernalization on the Development of Stripe in Barley.\(^1\)—Classification studies of North American barley varieties required the growing of a winter barley nursery at the experiment station in Aberdeen, Idaho, in 1944. As this nursery was to be planted in the spring, two series of seed were prepared and one of them was vernalized. The seed for the planting came from a classification nursery grown at Raleigh, North Carolina, in 1943. The seed to be vernalized was placed on blotters in Petri dishes in a refrigerator at the Plant Industry Station in Beltsville, Maryland, on February 23, 1944, where the vernalization was under the supervision of Dr. G. A. Wiebe. Sufficient water was added to keep the barley moist. The

TABLE 1.—Percentage of striped plants or culms developing from vernalized and nonvernalized seed lots

		Estimated percentage of striped plants ^b Percentage of striped culms at harvest,						
Barley strains	C.I.a No.	Jun	ie 1 6	Jun	e 28		ly 29	
	110.	Non- vernal- ized	Vernal- ized	Non- vernal- ized	Vernal- ized	Non- vernal- ized	Vernal- ized	
Hooded:								
Brugh 76	6477	trace	2	5	45	. 9	62	
Huga	6998	0	5	0	20	1	23	
Tucker	7039	0	5	trace	30	4	48	
Missouri Early Beardless	6051	trace	10	trace	30	6	42	
Hooded 16	6574	0	5	trace	10	1	15	
Iredell	6571	2	15	10	50	9	60	
Rough Awned: Wintex	6127	trace	20	5	50	6	45	

a C.I. refers to accession number of the Division of Cereal Crops and Diseases.

b Seed not space planted, therefore exact counts impossible.

moisture content was kept constant during a vernalization period of 38 days or until April 1. The temperature in the refrigerator was 33–34° F. from February 23 until March 15, and 31–32° F. from March 16 until April 1. At the end of the vernalization period some barley kernels had roots just breaking through the coleorhiza, others had roots from a few mm. to 2 cm. long. After April 1 the vernalized seed was dried at room temperature for 3 days and then mailed to Aberdeen, Idaho, where it was planted on April 8.

In the field, paired 10-foot rows of vernalized and nonvernalized seed of the same barley strain from the same seed source made direct comparisons possible. During the summer barley stripe (*Helminthosporium gramineum* Rabh.) was observed on a few of the winter barleys. At the beginning of June the vernalized rows of 7 barley strains had typical stripe infection

 $^{^{1}\,\}mathrm{Investigations}$ supported in part by a grant from the Wisconsin Alumni Research Foundation.

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while no stripe was noticeable on the nonvernalized rows of the same barley strains. Later in the season there was a definite difference in the amount of stripe on the vernalized and nonvernalized rows. The observations made during the growing season and the counts on the healthy and striped plants at harvest in the latter part of July are given in table 1.

The vernalization process favored the development of stripe. It is possible that the mycelium from diseased seeds infected the healthy seedlings. These results indicate that it might be possible to develop a technique for testing varietal resistance to barley stripe on the basis of a vernalization procedure.—EWERT ÅBERG, University of Wisconsin and Bureau of Plant Industry, Soils, and Agricultural Engineering, Plant Industry Station, Beltsville, Md.

Control of the Gall Disease of Gypsophila Caused by Phytomonas gypsophilae (Brown) Magrou.\(^1\)—The gall disease on newly grafted Gypsophila paniculata caused by Phytomonas gypsophilae (Brown) Magrou\(^2\) (Bacterium gypsophilae Brown\(^3\)) was adequately described by Brown\(^3\).\(^4\)

In the last 4 years several New Jersey growers of this plant have experienced increasing losses from the disease. Under New Jersey conditions, well-developed galls appear within 2 weeks after the plants are grafted. The galls appear mainly on the upper cut surface of the rootstocks in the region where the scion is inserted. They are soft and nodular and vary in diameter to 4 cm. As a result of the excessive growth of the galls, the scions fail to form a strong union with the rootstock and most of the plants die within a month. Where a gall develops along the inside slit in the root, the scion commonly is pushed completely away from the root, thus resulting in the death of the plant.

Control Measures. Brown⁴ recommended dipping gypsophila roots to be used for understock for $1\frac{1}{2}$ to 2 min. in a 1–1000 mecuric chloride solution in order to kill the gall bacteria that may be present on the surface. No injury to the roots was reported.

The same dip was tried by the author in June, 1942, and May, 1944, on several hundred newly grafted gypsophila plants but it caused considerable damage. A suspension of calomel made by dissolving $1\frac{1}{2}$ oz. gum arabic in a gallon of water and adding 2 oz. calomel also caused injury. Apparently, in the confined areas of the frames in which newly grafted plants are placed, the mercury still adhering to the dipped plants volatizes in sufficient amounts to cause considerable injury.

In May, 1944, and at periodic intervals throughout the summer and early fall, the value of other materials was determined. As a result it has been

1 Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Dept. of Plant Pathology.

2 Bergey's Manual of Determinative Bacteriology. Fifth Edition, p. 210. Williams & Wilkins, Baltimore. 1939.

³ Brown, Nellie A. Another gall-forming bacterium. Phytopath. 22: 924-925. 1932.
 ⁴ Brown, Nellie A. A gall similar to crown gall produced on Gypsophila by a new bacterium. Jour. Agr. Res. [U. S.] 48: 1099-1112. 1934.

found that a 2-min. dip of newly grafted plants in a calcium hypochlorite solution controls the disease without injuring the plants. Ordinary calcium hypochlorite sold in grocery and drug stores as chloride of lime was used at concentrations ranging from 2 to 12 oz. of the powder per gallon of water. Concentrations from 2 to 6 oz. per gallon caused no plant injury and still controlled the disease. The 12-oz. per gallon rate injured the foliage and was discontinued in later tests.

The preparation of the calcium hypochlorite solution is simple. The day before the solution is to be used, dissolve the powder in water and pass the solution through filter paper.

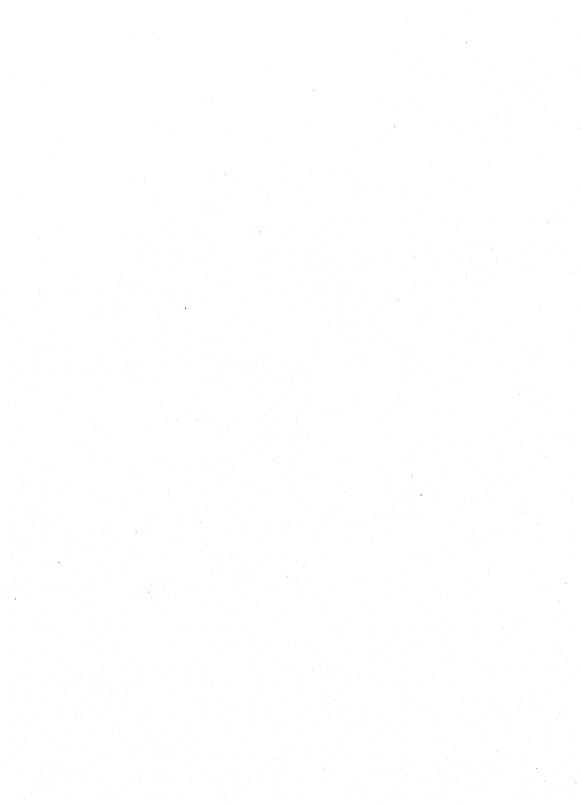
Table 1 presents the data collected from three typical tests during 1944.

TABLE 1.—Calcium hypochlorite dip to control gall disease of grafted gypsophila

D-4- 0- 11-1		Tre	eated plants	Co	Control plants		
Date treated	Oz. chemical - per gal. H ₂ O	No.	Per cent healthy	No.	Per cent healthy		
July 15	6	25	100.00	25	4		
Aug. 25	6 3	$125 \\ 125$	99.92 97.60	$125 \\ 125$	$\begin{array}{c} 25 \\ 36 \end{array}$		
Sept. 16	$rac{4}{2}$	$125 \\ 125$	99.00 98.00	$250 \\ 250$	50 60		

These data clearly indicate that the gall disease can be controlled by dipping newly grafted gypsophila plants for 2 to 3 min. in a calcium hypochlorite solution made by dissolving from 2 to 6 oz. of the powder in a gallon of water.

Among the materials tested and discarded because they caused plant injury or failed to control the disease were the 1-1000 mercuric chloride solution and calomel suspension mentioned; U. S. Rubber Company, Naugatuck Chemical Division Compound 604; and phenyl mercury salycilate dip, which is made by dissolving 1 part of the chemical in 400 parts of water.—P. P. PIRONE, New Jersey Agricultural Experiment Station, New Brunswick, N. J.



CULTURE TYPES AND PATHOGENICITY OF ISOLATES OF CORTICIUM SOLANI¹

BYRON R. HOUSTON 2,3

(Accepted for publication October 9, 1944)

INTRODUCTION

The fungus Corticium solani (Prill. and Del.) Bourd. and Galz. (C. vagum Berk. and Curt.) (Rhizoctonia solani Kühn) is known to be present in all parts of the world where its host plants are extensively grown. In California it causes serious losses in many of the common commercially grown crops including alfalfa, bean, cotton, cowpea, potato, and sugar beet. With the use of these crops in a system of rotation, it becomes important to know the specialization in relation to pathogenicity within the strains of this fungus.

This investigation was undertaken to increase our knowledge of the parasitism and cultural characters of isolates of the fungus obtained from agriculturally important hosts.

At present the species Corticium solani includes a group of fungi heterogeneous in nature with reference to their growth characters and their ability to produce disease. Studies of the morphologic characters of C. solani by Duggar (9), Le Clerg (16), Matsumoto (20), and Rosenbaum and Shapovalov (31) have shown that there may be slight or occasionally somewhat more pronounced morphologic differences between individual isolates. However, for the separation of cultural types the most desirable method found in this investigation was to use the differences shown by the isolates in their growth characters, pathogenicity, and physiology. In his textbook on diseases of plants, Julius Kühn in 1858 described and named Rhizoctonia solani as a fungus causing a disease of potatoes and thus distinguished it from the species R. violacea with which it apparently had been previously combined. An extensive review of early literature has been given by Duggar (9) and Peltier (24) and will not be included here.

Investigations on racial specialization have been undertaken by several workers including Duggar (9), Edson and Shapovalov (12), Gratz (13), Le Clerg (17, 18, 19), Matsumoto (20), Monteith and Dahl (21), Peltier (24), Sanford (32, 33), and Storey (34). In 1915 Duggar (9) made an elaborate study of the common *R. solani* and concluded that there was little marked specialization. Peltier (24) found no outstanding strain specialization in pathogenicity but noted differences in cultural characters. In 1917 Rosenbaum and Shapovalov (31) identified a new strain of *Rhizoctonia*

² Assistant Professor of Plant Pathology and Assistant Plant Pathologist in the Experiment Station, University of California.

³ The writer wishes to acknowledge his indebtedness to Dr. M. W. Gardner for his advice and criticism throughout these investigations and for editing the manuscript.

¹ The work herein reported was part of a thesis presented to the Graduate School of the University of California, September, 1939, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

occurring on potato on the basis of the type of lesions produced by it on the potato stems, its growth and sclerotial characters, and the different cell size noted when it was compared with their other isolates from potato. Matsumoto (20) in 1921 separated isolates of *R. solani* into several strains and suggested a possible specific rank for one of them. Le Clerg (16) first reported pathogenically distinct strains on potato and sugar beet; however, he later found (17, 18, 19) that some potato isolates were pathogenic to sugar beet and that most sugar-beet isolates were pathogenic upon potatoes. Storey (34) reported that some strains had a wide host range whereas others possessed a more selective parasitism.

From the investigations of Müller (22), Prillieux and Delacroix (25), Rolfs (29), Ullstrup (35) and others, it can be concluded that there is a definite connection between the vegetative stage, *Rhizoctonia solani*, and the perfect stage, *Corticium solani*, so the latter name should be used in preference to the former.

The correct nomenclature of this fungus has long been in controversy. The English and American pathologists have used the name *Corticium vagum* Berkeley and Curtis, whereas the continental European workers refer to the same fungus as *Corticium solani* Prillieux and Delacroix. The name *Rhizoctonia solani* Kühn is still commonly used in the literature.

In 1891 Prillieux and Delacroix (25) reported the occurrence of the basidial stage of a fungus on potato stems. The white mycelial growth, which could be easily removed, extended up the stem for several centimeters and did not penetrate the epidermis. This mycelial mat produced basidia, each with four sterigmata bearing smooth hyaline basidiospores. Under the system of classification used by them, the fungus was named Hypochnus solani, a new species, but they did not at that time connect this stage with the vegetative stage of the fungus, Rhizoctonia solani. However, from their drawings, descriptions, and measurements, it appears they were working with this organism.

Rolfs (29) observed the basidial stage of this fungus in 1903 on the leaves and stems of potatoes while working with the Rhizoctonia disease. He was able to germinate the spores and prove the connection between this stage and the vegetative stage pathogenic on the tubers and stems. He sent specimens of this fungus to Burt at the Missouri Botanical Gardens who referred it to Corticium vagum Berkeley and Curtis which had been described by the English mycologist Berkeley (2) in 1873. This name was originally applied by Berkeley to a specimen of a saprophytic fungus producing basidiospores on a thin hymenial layer on pine bark sent to him by Curtis from South Carolina. No connection between this and the vegetative state R. solani was proven. Burt (5), in comparing this with the specimen from Rolfs, noticed slight differences in the size and shape of the basidiospores, and thus named Rolf's fungus C. vagum B. and C. var. solani Burt. Vegetative stages of the two were not compared at this time. Rolfs accepted Burt's classification of the fungus and Hypochnus solani became a synonym. Most

American and English workers have accepted this until rather recently. In continental Europe the name Hypochnus solani was accepted until 1911 when Bourdot and Galzin (3) recognizing the difference in the systems of nomenclature used by Prillieux and Delacroix and by Burt (5) made the new combination Corticium solani. This latter name is now preferred by English and some American mycologists as well as by those in continental Europe, and also was used by Weiss (37) in his revision of the Check List of Diseases of Economic Plants in the United States. The complete citation, Corticium solani (Prill. and Del.) Bourd. and Galz., as suggested by Weiss, seems to be the correct one to use for this fungus. Recently, however, Rogers (28) has included Corticium solani and several other related fungi under the binominal Pellicularia filamentosa (Pat) Rogers.

SOURCE OF ISOLATES

With differences occurring between isolates from the different hosts and even between those from the same host, it is necessary, in order to study differences, that many isolations be made from each important host. The 52 isolates listed in table 1 and used in this study were obtained primarily from California and were selected from 260 isolations from 15 different crop plants in widely separated regions of the State. The placing of the isolates in the cultural groups A, B, and C is discussed in the following section. In the selection of the isolates a careful comparison of cultural characters was made of those from the same host and same locality, and only those with differences were selected. Also, preliminary pathogenicity trials were made on all isolates, and those from the same immediate locality, unless they appeared very different in culture, were found to have the same degree of pathogenicity on the same hosts. In this way, it is felt that a representative group from the more important hosts was selected. Before attempting a comparison of the isolates, pure cultures of each were assured by obtaining hyphal-tip cultures. All isolates having the prefix number 24 were hyphal tip cultures and all those having the prefix number 35 were single or multiple basidiospore cultures.

CULTURAL CHARACTERS

When growing on an agar medium there were some striking growth differences which were used to separate the isolates into several groups. Matsumoto (20) used the intensity of the darkening of the culture medium as a basis on which to separate the isolates studied by him into three groups. However, since this character is variable depending upon the type of culture medium employed and the environmental conditions, it must not be considered as one of the more important characters of growth. The striking features of growth were the presence or absence of a stroma-like layer of mycelium on the surface of the medium, the color of this layer, the relative amount of aerial mycelium produced, the abundance, type and color of the selerotia, and the growth rate.

TABLE 1.—Source and culture types of isolates of Corticium solani used in the pathogenicity trials

No. and culture type of isolate	Host from which isolated	Effect on host
$Type\ A$		~ .
24- 9	Bean, Lima	Stem_canker
24-49	Bean, Mung	do
24-52	Bean, Pink	do
24-21	Cotton	Damping off
24-22	do	do
24-29	do	₫ο
24-30	do	do
24-32	do	do
24–18	Cowpea, Blackeye	Stem_canker
24-86	_ do	do
24-82	Fenugreek	Damping off
24-42	Potato	Stem canker
24-14	Rhubarb	Petiole rot
24-8	Spinach	Damping off
24-85	Squash	Crown rot
24- 1	Sugar beet	Damping off
24-11	do	do
24-15	do	Dry-rot canker
24-35	do	do
24-38	do	do
24-43	do	do
24-48	do	đo
24-80	do	do
24-81	do	do
24- 7	Tomato	Damping off
24-83	do	Fruit rot
$egin{array}{c} Type\ B \ 24-\ 3 \end{array}$	Sugar beet	Dry-rot canker
24-17	do	do
24-24	do	do
24-27	do	do
24-34	do	do
24-36	do	do
24-37	do	do
24-40	do	do
24-45	do	do
$Type\ C$.70.70	
24-84	Alfalfa	Crown rot
24-12	Asparagus	Stem rot
24-76	Bean, Pink	Stem canker
35- 4	Celery	Basidiospore group
24-4	Potato	Tuber sclerotium
24–13	do	Stem canker
24-19	do	Tuber rot
24-28	do	do
24-51	\mathbf{do}	Tuber sclerotium
24-69	\mathbf{do}	do
35- 2	do	Basidiospore group
24- 6	Strawberry	Root rot
35- 1	Tomato fruit	Basidiospore group
35- 7	do	Single basidiospore

These characters were studied in 100 isolates growing at a constant temperature of 25° C. on a potato-dextrose agar of pH 6.8. The isolates were rather easily separated into three groups, here designated culture types A, B, and C.

Culture type A was characterized by: (1) a heavy stroma-like layer on the surface of the medium, at first almost white and later becoming Ridgway's (27) pale drab gray to light drab. This surface growth often was convoluted. A light cinnamon drab, multihyphal strand growth may be produced on the surface of this layer; (2) natal brown sclerotia, appearing as slightly raised areas of the surface growth, often radiating out from the point of inoculation and not typically small isolated bodies except those produced where the aerial mycelium grew in contact with the sides of the culture dish. This latter type was typically flat and not globular; (3) aerial mycelium sparse and white to cinnamon; (4) a rapid growth rate (1.0 to 1.8 mm. per hour); (5) often a pronounced darkening of the culture medium.

Culture type B was characterized by: (1) no pronounced stroma-like layer on the surface of the medium; (2) few, natal brown, globular sclerotia, one to four mm. in diameter, and commonly formed by the aerial mycelium when in contact with the sides of the culture dish, or suspended in the air; (3) very abundant aerial mycelium, pale olive buff to sayal brown, and forming a solid mass which usually grew well above the upper portion of the culture medium in a test tube slant; (4) a moderate growth rate (0.6–0.8 mm. per hour); (5) no darkening of the culture medium.

Culture type C was characterized by: (1) a slight stroma-like layer on the surface of the agar, at first vinaceous buff and becoming wood brown with age; (2) sclerotia natal brown, globular, with a very irregular surface, and often united into groups as large as 15 mm. in diameter. Individual sclerotia were one to five mm. in diameter and scattered over the surface growth; (3) aerial mycelium very sparse or lacking; (4) a moderate to slow growth rate (0.5 to 0.7 mm. per hour), (5) none to moderate darkening of the culture medium.

Representative isolates are in figure 1.

Although there were occasional isolates that appeared to be of a type between two of the defined types, as might be expected in such a heterogeneous group of fungi, most isolates could readily be placed in their respective group and this scheme affords an easy and workable means of separation. It seems to be usable for those isolates of *Corticium solani* that are parasitic upon the common commercially grown hosts in California and for all isolations that were made in the course of this study.

THE EFFECT OF TEMPERATURE ON RATE OF GROWTH

The effect of temperature on the rate of mycelial growth of a number of isolates was studied. Petri dishes of uniform size were sterilized and 10 ml. of sterile potato-dextrose agar poured into each dish. Dishes were inoculated with mycelium inoculum obtained by cutting discs 2 mm. in diameter from a point approximately 1 cm. behind the advancing margin of colonies growing on the agar, and placing the discs in the center of the poured dishes. In each of two trials four dishes of each isolate were placed at the following temperatures: 4°, 10°, 16°, 22°, 25°, 28°, 31°, 34°, and 40° C. The more

rapidly growing isolates advanced to the edges of the dishes in about 50 hours, so a 48-hour measurement was used in their comparison.

The isolates could be separated into three groups based upon their average growth rates and their optimum and maximum temperatures. Nine of the twenty-five isolates studied were selected as representative and are shown graphically in figure 2. The isolates 24–1, 24–7, 24–8, 24–9, and

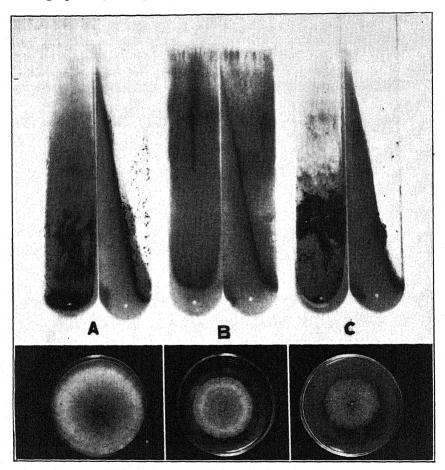


Fig. 1. Representative isolates of the three culture types of *Corticium solani*. Type A on the left, B in center, and C on the right. Above: Top and side views of the isolates grown for 2 weeks on potato-dextrose agar slants. Note differences in aerial growth and sclerotial production. Below: Diameter growth of the isolates after 48 hours at optimum temperatures.

24-25 can be combined in one group with an optimum temperature of 28° C. and with a 48-hour growth of from 30 to 60 sq. cm. Isolates 24-7 and 24-9 are respectively the slowest and most rapidly growing isolates of this group. Other isolates of this group, when growth area was plotted, fell between 40 and 60 sq. cm. The isolates comprising this group are of the cultural type A (Table 1). Isolates 24-3 and 24-26 represent culture type B, and

have an optimum temperature of 28–29° C. and a range of growth at 48 hours of 10 to 20 sq. cm. Isolates 24–62 and 24–28 represent culture type C, and their optimum temperature was from 24° to 26° C. and their maximum temperature was 33° C. as compared to a maximum of 40° C. for the other groups. Rarely did an isolate from one group fall within the growth limits of another, so that growth rate character could be used in conjunction with other growth characters in classifying an isolate.

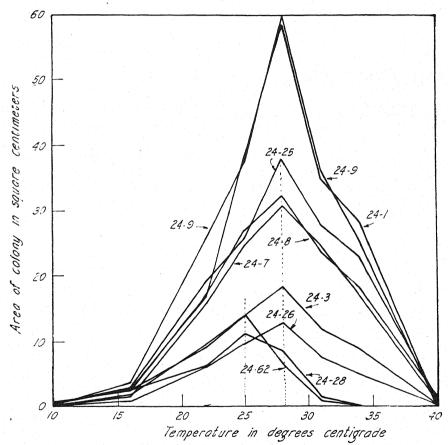


Fig. 2. The average growth on potato-dextrose agar of nine isolates of Corticium solani after 48 hours at nine different temperatures. Isolates 24–1, 24–7, 24–8, 24–9, and 24–25 are of culture type A; 24–3 and 24–26, culture type B; 24–28 and 24–62, culture type C. Types A and B have the same optimum and maximum temperatures but vary in rate of growth. Type C is distinct in that its optimum and maximum temperatures are below those of the other groups.

These results generally agree with previous work that has been done on different isolates of this fungus. Matsumoto (20) found the optimum temperature to be about 24° C. Müller (22) reported a temperature range of 4.5° to 30.8° C. with an optimum at 24° C. for the *Hypochnus solani* that he investigated. For the isolates he tested, Le Clerg (19) reported the optimum temperature for the sugar-beet isolates as 25° to 30° C. and for the

potato isolates as 20° to 25° C., but the growth rates were very similar at optimum temperatures. In figure 2, the growth of isolates 24-3 from sugar beet was approximately the same as Le Clerg's average for his sugar-beet isolates and the growth of isolate 24-62 from potato was about the same as that for his potato isolates. Walker (36), in working with an isolate of Rhizoctonia solani from cotton, found that the optimum temperature for growth was between 27° and 29° C., with the maximum growth being about the same as that of isolate 24-1 (Fig. 2). Monteith and Dahl (21), working with grass and potato strains, found wide variation in growth rates between the potato isolates, with the optimum growth between 25° and 30° C. and in most cases nearer 25° than 30°. Their curves for the growth of the grass isolates are very similar to the curves in figure 2 for isolates 24-1 and 24-9, except that their isolates grew about equally well at 25° and 30° C., the flat topped growth curve being only slightly higher at 30° C. If they had had a trial at 27° or 28° C., the curve might have been very similar to the curve obtained in this study for isolates 24-1 and 24-9.

MATERIALS AND METHODS USED IN PATHOGENICITY STUDIES

Differences in pathogenicity of isolates of Corticium solani have been noted by a number of investigators (10, 12, 17, 18, 19, 23, 30). In this study the trials were limited to inoculations by isolates from previously mentioned important hosts upon alfalfa (Medicago sativa L.), pink bean (Phaseolus vulgaris L.), cotton (Gossypium hirsutum L.), potato (Solanum tuberosum L.), spinach (Spinacia oleracea L.), sugar beet (Beta vulgaris L.) and tomato (Lycopersicum esculentum Mill.). In all of the experiments, except those involving field inoculations on growing sugar beets, two-thirds cu. ft. of steam-sterilized soil in flats was infested by adding to it giant cultures of the isolates grown for approximately one month upon 30 g. by dry weight of sterile oat grains. This amounted to the addition of approximately 0.1 per cent of organic matter to the soil. A like amount of oat culture subsequently steam-sterilized was added to the check flats. After addition of the inoculum, the soil was moistened and stirred daily for 6 to 8 days before planting. All experiments were in duplicate and were repeated twice.

Isolations were made from the diseased plants in the infested soil in each case and over 99 per cent of all isolations were *Corticium solani*. All proved to be the same isolate that was used in the inoculation.

PATHOGENICITY ON SEEDLINGS OF FIVE CROP HOSTS

The results of the pathogenicity trials on the seedlings of alfalfa, cotton. spinach, sugar beet, and tomato are in table 2. The total percentage of disease was a summation of the percentage of post-emergence and pre-emergence damping-off. This summation seemed justifiable because the isolates differed only slightly in their pathogenicity to seedlings of different ages.

The isolates of type A are, in general, very pathogenic on seedlings of the five hosts regardless of the host from which they were originally isolated.

 ${\bf TABLE~2.} {\it --The~relative~pathogenicity~of~isolates~of~the~three~culture~types~of~Corticium~solani~on~seedlings~of~five~hosts$

No. and cul-	Source of —		Total pe	ercentage of	diseasea	
ture type of isolate	isolate	Alfalfa	Cotton	Spinach	Sugar beet	Tomato
$Type\ A$						
24- 9	Bean, Lima	100	98	96	90	78
24-49	Bean, Mung	100	100	83	57	90
24 - 52	Bean, Pink	98	96	85	78	63
24-21	Cotton	26	12	20	26	0
24-22	do	100	90	78	84	57
24-29	do	84	89	73	57	0
24-30	do	14	42	29	52	0
24-32	do	92	82	82	81	16
24-18	Cowpea, Blackeye	100	97	89	94	28
24-86	do	100	100	83	70	70
24-82	Fenugreek	78	72	74	57	13
24-42	Potato	100	89	79	98	98
24-14	Rhubarb	100	91	74	100	50
24-8	Spinach	54	62	13	35	8
24-85	Squash	100	82	86	88	94
24-1	Sugar beet	100	94	54	98	98
24-15	do	100	74	50	96	100
24-35	do	99	82	68	92	95
24-38	do	95	73	77	97	34
24-43	do	84	77	59	74	70
24-48	do	100	100	72	82	65
24-80	do	96	93	$7\overline{2}$	95	46
24-81	do	100	66	41	76	79
24-11	do	2	0	0	i	0
24-7	Tomato	88	100	100	96	98
24-83	do	14	100	23	21	26
Tuna P	Average	82	79	64	73	53
$Type\ B$	Cramon book	. 0	0	0	16	0
24 3 2417	Sugar beet	31	0	0	0	$\frac{0}{0}$.
24-24	do do	14	11	0	1	10
24-24	do	1	0	0	1	0
24-27		1	0	8	0	0
24-34 24-36	do	1	0	0	$\overset{0}{2}$	0
	do				3	0
24-37	do	39	1	0	13	
24-40	do	0 0	$\frac{0}{22}$	0	$\frac{13}{28}$	19
24–45	đo	-			7	0
$Type\ C$	Average	10	4	1		3
24-84	Alfalfa	27	5	16	0	0
24-76	Bean, Pink	5	0	10	0	0
35-4	Celery	0	17	0	0	16
24- 4	Potato	14	12	0	0	0
24-13	do	9	0	13	28	0
24-19	do	2	0	4	0	0
24-28	do	1	0	0	25	0
24-51	do	19	13	15	11	0
24-69	do	2	12	0	0	0
35- 2	do	2	0	0	19	0
24- 6	Strawberry	ī	Ö	3	0	Ŏ
35- 1	Tomato	9	Ŏ	0	10	$\overset{\circ}{2}$
35- 7	do	Ö	$\overset{\circ}{2}$	ŏ	0	$1\overline{4}$
	Average	7	5	5	7	2
Control	Sterile soil	0	0	0	0	0

a The total percentage of disease was based upon the surviving stand of seedlings in

There were a few isolates, particularly 24–21 and 24–30 from cotton and 24–8 from spinach, which were only moderately pathogenic. Isolates 24–11 and 35–11 from sugar beet caused little damping-off of any of the hosts. Isolate 24–11 was originally isolated from a sugar-beet seedling and 35–11 is closely related to it in that it was obtained from the resultant growth of a group of basidiospores dropped onto agar from the hymenium of 24–11. Isolates of type A were commonly found in nature infecting sugar beet, bean, cowpea, cotton, and spinach. Occasionally they were obtained from potato stem lesions.

The isolates of type B, in contrast to those of type A, have only slight seedling pathogenicity, occasionally being pathogenic to seedlings of one host but not to those of another. This might be expected of this group since isolates of this kind were not found on seedlings, and the writer has never isolated one of this type from natural infections on any host other than sugar beet.

Isolates of type C proved to be somewhat more pathogenic than those of type B, but at the most they were only slightly pathogenic on seedlings. They were commonly found infecting potato, producing sclerotia on tubers and lesions on stems and stolons. Very seldom have they been isolated from naturally infected seedlings of the hosts studied. Most isolates obtained from the naturally occurring basidiospore stage have been of this type.

In general, isolates of type A are capable of attacking the seedlings of all the hosts studied, whereas, those of type B are relatively nonpathogenic, and those of type C are somewhat variable in their degree of pathogenicity but are not severely pathogenic on seedlings. On alfalfa the average percentage of damping-off produced by all of the isolates comprising each of the three culture groups A, B, and C was 82, 10, and 7 respectively. The disease produced on the other hosts by the three culture types was approximately in this same ratio.

The susceptibility of the five hosts varied somewhat in that tomato was usually more resistant to attack than were any of the others. This was especially true of those isolates with only a moderate degree of pathogenicity. Spinach was somewhat more resistant than alfalfa, cotton, or sugar beet, and alfalfa was generally the most susceptible.

There was no damping-off of the seedlings in any of the control flats of steam sterilized soil.

PATHOGENICITY ON PINK BEAN

Rhizoctonia lesions on bean plants in the field may be observed at almost any stage of growth. The surface scabbing of the older stems, however, is apparently a result of a partial healing and recovery from an earlier infection. The first record of Rhizoctonia injury to bean seedlings was given by Atkinson (1). He reported that the fungus caused damping-off of bean seedlings and attacked plants that were 6 to 10 inches high. In 1901 Duggar and Stewart (10) reported this fungus as the cause of a stem rot of red kidney beans in the field. Since that time there have been many reports on its occurrence on beans.

 $\begin{tabular}{ll} TABLE 3. — The relative pathogenicity of isolates of the three culture types of Corticium solani on pink bean \end{tabular}$

No. and culture type of isolate	Source of isolate	Emer- gence	Diseased seedlings	Injury rating ²	Points of injury listed in order of importance
		$Per\ cent$	Per cent		
Type A $24-9$ $24-49$ $24-52$	Bean, Lima Bean, Mung Bean, Pink	28 58 60	100 98 88	3 3 3	Primary root, hypocotyl do do
$24-21 \\ 24-22 \\ 24-29$	Cotton do do	96 38 98	$\begin{array}{c} 0\\100\\100\end{array}$	0 3 3	Scabbing on surface Primary root, hypocotyl do
24-30 24-32 24-18	do do Cowpea, Blackeye	100 84 44	70 100 92	2 3 3	Hypocotyl Primary root, hypocotyl do
24-86 24-82 24-42	đo Fenugreek Potato	78 64 18	$ \begin{array}{c} 91 \\ 82 \\ 100 \end{array} $	3 2 3	do Hypocotyl Primary root, hypocotyl
24-14 $24-8$ $24-85$	Rhubarb Spinach Squash	22 92 56	100 0 100	3 0 3	do Scabbing on surface Primary root, hypocotyl
24 1 2415 2435	Sugar beet do do	16 46 74	100 100 100	3 3 3	do do do
24–38 24–43 24–48	do do do	80 80 94	100 98 100	3 3 3	do Hypocotyl, primary root do
24-80 24-81 24-11	do do do	88 34 84	100 100 8	3 3 1	do Primary root, hypocotyl Hypocotyl
24- 7 24-83	Tomato do	26 20	100 100	3	Primary root, hypocotyl do
Type B	Average	61	86	2.6	
$\frac{1}{24}$ $\frac{1}{3}$	Sugar beet	98	10	1	Hypocotyl
24-17	do	96	õ	0	No injury
24-24 24-27	do do	94 98	5 0	1 0	Hypocotyl
24-27	do	98	60	1	No injury Hypocotyl
24-36	do	98	ő	0	No injury
24-37	do	98	60	1	Hypocotyl
24-40	do	98	0	0	No injury
24-45	do	98	18	1	Hypocotyl
Type C	Average	97	17	0.6	
24-84	Alfalfa	98	0	0	No injury
24-76	Bean, Pink	98	0	0	do Humanatul
35- 4 24- 4	Celery Potato	$\begin{array}{c} 96 \\ 100 \end{array}$	10 0	$\begin{array}{c} 1 \\ 0 \end{array}$	Hypocotyl No injury
24-13	do	96	0	0	do
24-19	do	62	6 <u>0</u>	$\overset{\circ}{2}$	Hypocotyl
24-28	do	100	0	0	No injury
24-51	do	92	0	1	Hypocotyl
24-69	do	96	0	0	No injury
35- 2	do	98	0	0	do
24- 6 35- 1	Strawberry Tomato	$\begin{array}{c} 100 \\ 100 \end{array}$	0	0	do do
35- 7	do	97	0	0	do
	Average	95	5	0.3	
Control,	, sterile soil	100	0	0	No injury

In this study certain isolates were capable of attacking the primary root of the young plant both before and after emergence of the cotyledons. This attack was usually associated with swelling and distortion of the hypocotyl and with hypocotyl lesions. Other isolates caused no injury to the primary root, but severely injured the hypocotyl. Others produced no injury whatever. Since the infections varied as to their degree of severity, an injury



Fig. 3. The common types of lesion on pink-bean seedlings produced by isolates of Corticium solani. The large seedling on the left has typical hypocotyl lesions commonly produced by isolates of type A or occasionally B. The smaller seedlings on the right have the primary root, hypocotyl, cotyledon, and leaf lesions of the type often produced by isolates of type A. The seedlings were the same age when photographed, but the two on the right were greatly stunted because of the more severe injury.

rating of 0 for healthy to 3 for severely diseased was used in the classification of the diseased plants and as a means of rating the pathogenicity of the isolates. The rating was based upon the percentage emergence and the degree of injury to each plant at the end of four weeks. This seemed to be a somewhat more accurate measure of the pathogenicity than the percentage of disease alone. The results obtained from the inoculations on pink bean are summarized in table 3.

Isolates of type A with but few exceptions were strongly pathogenic on pink bean, in some cases causing severe pre-emergence injury to the primary root and hypocotyl. These few that produced slight injury were the same isolates that proved to be weakly pathogenic in the seedling trials. The isolates obtained from naturally infected beans and related hosts were predominately type A, so this group might be expected to be very pathogenic to beans.

Here again, the isolates of the culture types A and B can be separated on the basis of their ability to produce disease. Although some B isolates caused small hypocotyl lesions, none of them were strongly pathogenic and none produced pre-emergence injury or injury to the primary root (Fig. 3). Thus, the most pathogenic isolates of this group were given an injury rating of only 1 as compared to a rating of 3 for the majority of those in group A. Isolates of type B have never been obtained from naturally infected beans.

Isolates of type C were variable in their pathogenicity on beans but with only two isolates, 24–19 from potato and 24–83 from tomato, was there severe injury to the plants. It is of interest that isolate 24–52 originally obtained from a very small hypocotyl lesion on pink bean was non-pathogenic. Occasionally isolates of this type were obtained from naturally infected beans, but seldom did they produce appreciable injury either to the plant from which isolated or to reinoculated plants.

The average percentage of diseased seedlings produced by all individuals of each of the culture groups A, B, and C was 86, 17, and 5, respectively, with corresponding degrees of injury of 2.6, 0.6, and 0.3.

PATHOGENICITY ON POTATO

The attack of Corticium solani, then known as Rhizoctonia solani, on potato has been known in Europe since 1858 when Kühn (15) described the disease and named the fungus. In the United States Duggar and Stewart (10) first reported the Rhizoctonia disease of potato from New York in 1901. Differences in pathogenicity of potato isolates have been noted by Dana (6), Drayton (7), Duggar (9), Gussow (14), Le Clerg (18, 19), Rolfs (30), Rosenbaum and Shapovalov (31), Sanford (33), and others.

For inoculation tests, seed tubers of the White Rose variety free from Corticium sclerotia were surface sterilized with a 1 to 1000 solution of bichloride of mercury for $1\frac{1}{2}$ hours and planted in soil infested with the isolates. For each isolate a total of thirty tuber pieces each containing a single eye were used in a series of three replications.

The plants were grown for two months after which they were removed and the stems examined for lesions and sclerotia. The results of these trials are presented in table 4.

Most of the isolates of group A were pathogenic on the stems and stolons of potato but produced relatively few sclerotia on the tubers. A large majority of the stem lesions resulting from the attack by type A were more severe than those produced by the other two types and often resulted in

complete girdling at the point of infection (Fig. 4). The bean, potato, tomato, and sugar-beet isolates of type A were strongly pathogenic causing a high percentage of infection. The cotton and spinach isolates were somewhat less pathogenic. The isolates 24–30 from cotton and 24–8 from spinach produced no infections on the stems or stolons.

TABLE 4.—The relative pathogenicity and sclerotial production of isolates of the three culture types of Corticium solani on potato

No. and culture type of isolate	Source of isolate	Shoots emerged from 30 lber pieces	Diseased shoots	Sclerotial production	Degree of injurya
A COLUMN TO THE PARTY OF THE PA		Number	Per cent		
Type A					
24- 9	Bean, Lima	30	100	+	3
24-52	Bean, Pink	24	68	+	2
24-21	Cotton	25	10	0	1
24-22	do	26	6	0	1
24-30	do	26	0	0	0
24-18	Cowpea, Blackeye	22	60	0	2
24-42	Potato	20	61	+	3
24-8	Spinach	32	0	0	0
24- 1	Sugar beet	30	50	0	2
24-15	do	18	33	0	2
24-25	do	20	40	+	2
24-11	do	24	16	0	1
24 - 7	Tomato	34	100	. · · · · · ·	3
<i>m</i>	Average	25	42		
$Type\ B$	6	20	^	^	
24-3	Sugar beet	28	0	0	0
24-17	do	22	10	0	1
24-24	do	24	0	0	0
24-26	do	28	0	0	0
24-27	do	24	0	0	0
Tama C	Average	25	2		0.2
Type C 24- 4	Potato	21	18		1
24-13	do	22	40	++	$\frac{1}{2}$
24-13	do	20	10	++	1
24-19	dο	20 24	4	+	1
24-28	do	26	10	++	1
24-69	do do	$\frac{26}{24}$	12	++	
35- 2	do	26		++	1
35 7	Tomato	20 30	8	+++	1
99 /	Tomato	30	3	+	0
	Average	24	13		1.0
Control		30	0	0	0

a 0 = healthy; 1 = slightly diseased; 2 = moderately diseased; 3 = severely diseased with no chance of continued growth, or dead.

Isolates of type B from sugar beets in contrast to those of type A from the same host were weakly pathogenic. Only one isolate, 24–17, produced any infection, and this was mostly superficial causing no great injury to the stem. No sclerotia were produced on the tubers or stems by any type B isolate.

In nature the potato is the most common host for isolates of type C (Table 1). In the inoculation trials the isolates of this type were variable

in regard to their pathogenicity on potato but all produced some disease. The fact that this type produces large numbers of sclerotia on the stems and tubers (Fig. 4) undoubtedly explains the widespread distribution and constant association of this type with potato.

These results show that the isolates that are most pathogenic to seedlings, namely those of the A type, were also the most destructive to potato plants. The fact that the potato isolates of the C type produced more disease on potato than on the other hosts studied indicates a degree of host specialization for the group.

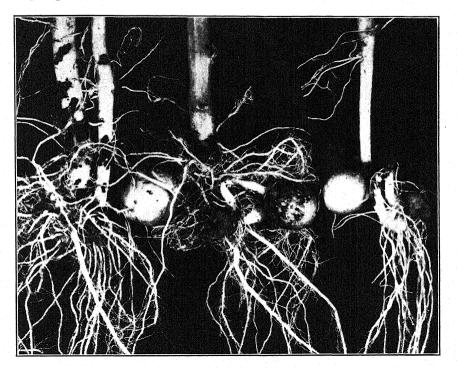


Fig. 4. The two potato plants on the left have the abundant sclerotia commonly produced by isolates of culture type C on the stems and tubers. Stem cankers were occasionally produced by this type. The plant on the right shows the typical stem girdling produced by isolates of type A. Two stems have broken off at the lesion.

PATHOGENICITY ON SUGAR-BEET ROOTS

The rotting of sugar-beet roots by Corticium solani after the plants are beyond the seedling state is of common occurrence and has been known for many years. Pammel (23) in 1891 described Rhizoctonia rot of sugar beets and named the fungus Rhizoctonia betae Kühn. Duggar (8) reported the occurrence of Rhizoctonia on sugar beets in America in 1899. In 1915 Edson (11) reported that R. solani was capable of attacking the sugar beet at any age without the presence of wounds. Edson and Shapovalov (12) reported a number of isolates of R. solani from sugar beet as being able to attack potato. Le Clerg (16) showed that sugar-beet isolates were patho-

genic on sugar-beet roots and seedlings while potato isolates were not. Later (17, 18, 19), he found that some isolates from potato were able to attack sugar beets. Peltier (24) found a sugar-beet isolate pathogenic to carnations. Wiant (38) tested two isolates from sugar beets and found them pathogenic on conifer seedlings, peas, cabbage, eggplant, pepper, and tomato.

This fungus is the cause of dry-rot canker, in certain regions the most important root rot of the sugar beet. The infection may be rather general over an entire field or limited to small circular areas where nearly 100 per cent of the plants may be killed during the season. The symptoms on the parts below ground may be of two types (Fig. 5). The more common type

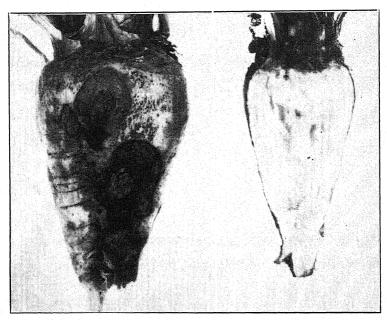


Fig. 5. The common symptoms produced by *Corticium solani* on sugar beets. On the left is the sunken concentric-ringed dry-rot canker. The beet on the right shows a sectional view of the crown-rotting phase.

is the more or less localized lesions of dry shrunken tissue each with concentric rings of lighter and darker brown dead tissue and with the internal tissue converted into a dry spongy mass in a definite pocket sharply delimited from the healthy tissue. Occasionally a large open canker is produced in which the dry diseased tissue cracks open widely, probably because of the continued increase in the circumference of the unaffected portion of the root. The other type is the crown infection resulting in the complete destruction of the crown tissues and the death of the leaves. Both kinds of infection may be produced by the same isolate.

During two seasons sugar beets growing in a field that was apparently free from natural infection were inoculated with 26 isolates of this fungus from various hosts. The fungus was grown on sterilized oats as previously described and when the beets in the field were from $1\frac{1}{2}$ to 3 inches in diameter, each was inoculated with 8 to 10 infested oat grains. The inoculum was placed in contact with or very near the uninjured root or sprinkled between the petioles of the leaves on the crown of the plant. In three randomized replications a total of 45 root and 45 crown inoculations were made with each isolate. During the growing season counts were made at monthly intervals and all dead beets staked so that they could be accounted for at the end of the season. The roots were dug after approximately five months at which time they were segregated into five classes, 0 to 4 (Fig. 6), as determined by the degree of injury. From a practical standpoint isolates pro-



Fig. 6. Sugar beets infected by *Corticium solani* following inoculation by placing the inoculum at the side of the injured roots. Examples of the four degrees of infection (1 to 4) used in the classification of diseased roots.

ducing an average injury rating of less than 0.8 can be considered as non-injurious.

Plants with previously injured roots were inoculated with several isolates, and with pathogenic isolates these plants died sooner than plants with uninjured roots. In no case did the isolates which failed to infect sound roots produce any disease other than a scabbing on the surface of the roots that were wounded. In such cases the wound healed very rapidly.

The results of the inoculation trials on sugar beets are in table 5. In the root inoculations, isolates of type A produced a high percentage of disease with the exception of the cotton isolate 24–21 which was mildly parasitic in these trials. In most cases the injury was severe, often resulting in the death of many of the plants within a month after inoculation. Eight of the eleven isolates produced over 95 per cent disease with corresponding degrees of injury greater than 2.9. This indicates the extreme virulence of

culture type A. Isolate 24–11, originally isolated from a sugar-beet seedling, was only moderately pathogenic on the older roots as compared with the other sugar beet isolates. The type A isolates that produced a high percentage of disease on the roots were also capable of invading the crown tissues. However, there was usually somewhat less infection produced by crown inoculation. The percentage of infection and degree of injury produced by isolates 24–29 and 24–11 were markedly low in the crown inoculations.

Isolates of type B also proved to be extremely pathogenic on sugar beet roots. The six isolates tested produced an average disease percentage of 97 and an injury rating of 3.0. Each isolate produced greater than 87 per cent disease. This virulence was outstanding as a point of distinction for these

TABLE 5.—The relative pathogenicity of isolates of the three culture types of Corticium solani on sugar beet roots and crowns

No. and		Root ino	culations	Crown in	oculations
culture type of isolate	isolate d	Average egree of injury ^a	Disease, per cent	Average degree of injurya	Disease, per cent
Type A		- 1.			
24- 9	Bean, Lima	3.4	100	3.5	100
24-52	Bean, Pink	2.2	82	2.2	76
24-21	Cotton	0.3	18	0.0	0
24-29	do	2.9	96	0.1	2
24-18	Cowpea, Blackeye	3.0	98	1.9	$6\overline{2}$
24-42	Potato	3.5	100	3.1	93
24-14	Rhubarb	3.6	100	2.9	93
24-1	Sugar beet	3.6	100	3.5	100
24-15	do	3.5	100	2.8	93
24-25	do	3.0	98	2.3	82
24-11	do	1.2	47	0.3	32 11
	QU.	1.4	±./	0.0	TT
	Average of grou	p 2.7	85	2.1	65
Type B		•			
24-3	Sugar beet	2.8	100	0.4	16
24- 5	do	2.5	93	0.2	9
24-17	do	2.4	87	0.6	20
24-24	do	3.2	100	0.3	13
24-26	do	3.1	100	0.4	16
24-27	do	3.7	100	0.8	29
	. 40	0.1	100	0.0	29
	Average of grou	р 3.0	97	0.5	17
$Type \ C \ 24-12$					
24-12	Asparagus	0.9	29	0.0	0
	Bean, Pink	0.1	4	0.2	7
24-13	Potato	0.3	13	0.0	0
24-19	do	0.8	24	0.1	2
24-51	do	0.5	22	0.1	2
24-64	do	0.2	11	0.3	11
35- 2	do	0.6	22	0.1	2
24- 6	Strawberry	0.6	22	0.3	11
24-10	Tomato	0.6	22	0.1	2
	Average of grou	n 0.5	19	0.1	4
Control	211CIASC OI SIOU		0	0.0	0

a 0 = healthy; 1 = slight infection; 2 = moderate infection; 3 = moderate-severe infection; 4 = severe infection.

isolates because of the fact that they were, from a practical standpoint, non-pathogenic on seedlings, including those of sugar beet. Also, when inoculated on the crown of the beet, isolates of this type were unable to infect the petioles and crown tissues, thus differing from most isolates of type A in this respect. Undoubtedly these are the reasons for the writer's failure to find isolates of type B commonly associated with natural seedling or sugar-beet crown infections. All of these isolates were obtained from the dry-rot canker stage of the disease on the sugar-beet roots.

The relatively low incidence of disease resulting from either crown or root inoculations with type C isolates sets them off as a distinct group differing from the other two groups in their ability to infect sugar beet. Since this type is that commonly associated with the black scurf disease of potato, these results agree with those of Le Clerg (16, 18) who found that most of the potato isolates were relatively nonpathogenic on sugar beet although an occasional isolate from potato stolons was able to readily infect sugar-beet roots. The type A potato isolate 24–42 was of this latter type and in these trials proved to be extremely pathogenic on sugar beets resulting in infections of 100 and 93 per cent in the root and crown inoculations, respectively.

DISCUSSION

The results of the pathogenicity trials clearly indicate that the fungus Corticium solani can readily be separated into pathogenically as well as culturally different strains. Some of these strains are limited in their attack to a certain host plant and over a period of years have been found constantly associated with this plant. Others, however, are capable of attacking a wide variety of plants and cannot be considered as being specific to any one particular host. In these trials the strain designated as type A was obtained from many hosts and is clearly a polyphagous strain showing no host specificity, whereas the strains designated as types B and C are highly specific as to hosts. The isolates of type B were obtained only from sugar beet roots, and those of type C were largely from potato and from basidiospores produced in nature.

Although type B occasionally produced some disease on hosts other than sugar beet in the inoculation trials, it has not as yet been found attacking any host other than this in nature. Besides being limited in pathogenicity to this one host, this strain invades only the enlarged root since it apparently cannot infect through the crown tissues nor is it pathogenic on sugar beet seedlings. Isolations from naturally infected beet roots have shown that isolates of group B are recovered only from infections originating below the crown.

Isolates of type C were obtained largely from potato and were somewhat variable in their ability to produce disease. They had little effect upon the seedlings of the hosts tested but showed a moderate degree of pathogenicity on potatoes. This was particularly true of those originally isolated from potato stem lesions or sclerotia. In general, isolates of this type compared

culture type A. Isolate 24–11, originally isolated from a sugar-beet seedling, was only moderately pathogenic on the older roots as compared with the other sugar beet isolates. The type A isolates that produced a high percentage of disease on the roots were also capable of invading the crown tissues. However, there was usually somewhat less infection produced by crown inoculation. The percentage of infection and degree of injury produced by isolates 24–29 and 24–11 were markedly low in the crown inoculations.

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24-11	do	1.2	47	0.3	11
Type B	Average of group	2.7	85	2.1	65
24- 3	Sugar beet	2.8	100	0.4	16
24- 5	do	2.5	93	0.4	9
24-17	do	$\frac{2.3}{2.4}$	87	0.6	20
24-24	do	3.2	100	0.3	
24-26	do	3.1	100		13
24-27	do	3.7	100	0.4 0.8	$\begin{array}{c} 16 \\ 29 \end{array}$
	Average of group		97	0.5	
Type C	Trorage or group	, 5.0	91	0.5	17
24-12	Asparagus	0.9	29	0.0	0
24-76	Bean, Pink	0.1	4	0.2	7
24-13	Potato	0.3	13	0.0	ò
24-19	đo	0.8	24	0.1	$\ddot{2}$
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Control			- 0	0.0	ō

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The results of the pathogenicity trials clearly indicate that the fungus Corticium solami can readily be separated into pathogenically as well as culturally different strains. Some of these strains are limited in their attack to a certain host plant and over a period of years have been found constantly associated with this plant. Others, however, are capable of attacking a wide variety of plants and cannot be considered as being specific to any one particular host. In these trials the strain designated as type A was obtained from many hosts and is clearly a polyphagous strain showing no host specificity, whereas the strains designated as types B and C are highly specific as to hosts. The isolates of type B were obtained only from sugar beet roots, and those of type C were largely from potato and from basidiospores produced in nature.

Although type B occasionally produced some disease on hosts other than sugar beet in the inoculation trials, it has not as yet been found attacking any host other than this in nature. Besides being limited in pathogenicity to this one host, this strain invades only the enlarged root since it apparently cannot infect through the crown tissues nor is it pathogenic on sugar beet seedlings. Isolations from naturally infected beet roots have shown that isolates of group B are recovered only from infections originating below the crown.

Isolates of type C were obtained largely from potato and were somewhat variable in their ability to produce disease. They had little effect upon the seedlings of the hosts tested but showed a moderate degree of pathogenicity on potatoes. This was particularly true of those originally isolated from potato stem lesions or sclerotia. In general, isolates of this type compared

with those of type A must be classified as very slightly pathogenic on all hosts other than potato.

The variation in pathogenicity is much greater when the isolates are grouped according to their original host than when grouped on the basis of culture type. Consequently, the culture type is often of more importance than is the host from which isolated when attempting a prediction of the possible pathogenicity and host range of an isolate. Because of this fact the generalization that all isolates from one host will or will not be pathogenic on another cannot be made. The fallacy of such a generalization is emphasized when a comparison of the pathogenicity of all the sugar-beet isolates is made. Such a group comprises isolates of A and B culture types both of which commonly affected sugar-beet roots and proved to be equally virulent when reinoculated onto the roots, but differed markedly in their ability to infect other hosts. Since both A and C isolates can attack potato and have been obtained from this host, it cannot be said in a general sense that potato isolates are nonpathogenic on hosts such as bean or sugar beet.

These results indicate that the cultural characteristics of an isolate of *Corticium solani* are more important in the prediction of the host specificity or lack of specificity of that isolate than is the host plant from which it was isolated. This is an important consideration in any area where a polyphagous strain occurs.

SUMMARY

From 260 isolations of *Corticium solani* from 15 different crop plants, 52 isolates were selected for pathogenicity studies. The selections were made on the basis of cultural characters and observed pathogenicity.

The isolates were separated into three culture types designated as A, B, and C based upon the following cultural characters: thickness and coloration of the stroma-like layer on the surface of the medium; size, abundance, and color of the sclerotia; abundance and color of the aerial mycelium; darkening of the agar medium; and growth rate. Isolates in group B were obtained only from sugar-beet roots in the field.

The effect of temperature upon the growth of 25 isolates was tested. The results were similar to those obtained for *Corticium solani* by other investigators. The majority of isolates had an optimum temperature of 28° C. and a maximum temperature of 42° C., and were variable in their rate of growth. The isolates of type C differed from this in that they had an optimum temperature of 25° C. and a maximum temperature of 33° C. This difference clearly sets this group off as a distinct temperature group. There was some overlapping of growth rates among the three groups so this method of separating them could be used only in conjunction with the other cultural characters.

The pathogenicity of 52 isolates was tested on alfalfa, cotton, pink bean, potato, spinach, sugar beet, and tomato.

Alfalfa seedlings proved to be susceptible to the attack of many of the isolates. In general type A was highly pathogenic to alfalfa, whereas B

and C were variable in virulence, but in no case highly pathogenic. The basidiospore isolates of type C were only slightly pathogenic. This is also true of isolate 24–11 of type A, which under certain conditions produced basidiospores on the inoculated host plants.

Cotton was susceptible to the same isolates that attacked alfalfa. The isolates of type A were the most destructive. Those of type B were practically nonpathogenic and those of type C were variable in pathogenicity.

Spinach seedlings were less susceptible than alfalfa or cotton. However, isolate 24–7 of type A was very destructive. The isolates of type A were moderately to severely pathogenic, those of type B were nonpathogenic, and those of type C were variable in their degree of pathogenicity but in no case were they severely pathogenic.

Tomato seedlings were killed by this fungus primarily in the pre-emergence stage. Isolates of type A were more variable in their attack on this host than on the others. Isolates of types B and C were nonpathogenic or only slightly pathogenic.

Sugar-beet seedlings were very susceptible to isolates of type A and to certain ones of type C. Others of type C and those of type B were only slightly pathogenic.

On pink beans, as on other hosts, the isolates of type A were very destructive. The isolates of types B and C were slightly pathogenic and produced symptoms on the beans which were different than those produced by the more pathogenic isolates.

The isolates of type A produced severe cankers on potato stems but only few sclerotia on the tubers. Isolates of type B produced no cankers or sclerotia, whereas those of type C produced a moderate number of cankers and many sclerotia.

Isolates of type A were able to infect growing sugar beet roots either through the crown or root, whereas isolates of type B could infect only through the root. This latter group could not infect sugar beet or other seedlings to any extent and is apparently a specialized group able to infect only the growing root of the sugar beet.

Prediction of the possible pathogenicity of any isolate of *Corticium solani* obtained from the more important host plants in California is more accurate when based upon the known pathogenicity of the culture type to which it belongs than when based upon the host from which it was isolated. Two isolates of different culture types having the same degree of pathogenicity on one host are not necessarily equally virulent in their attack upon another host.

Division of Plant Pathology, University of California, Davis, California

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SPECIALIZATION IN ERYSIPHE GRAMINIS FOR PATHOGENIC-ITY ON WILD AND CULTIVATED GRASSES OUTSIDE THE TRIBE HORDEAE¹

JOHN R. HARDISON²

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In previous papers (1, 2) it has been reported that most of the specialized races of *Erysiphe graminis* DC. studied from grasses in the tribe Hordeae and the genus *Poa* are not restricted in infection to the species of any one genus. This paper describes the pathogenicity of cultures from other grasses.

LITERATURE REVIEW

A general review of the literature dealing with host specialization in *Erysiphe graminis* and a detailed review of previous results with the fungus on grasses in the tribe Hordeae and the genus *Poa* have been presented (1, 2). Therefore, this review is restricted to the literature concerned with fungus cultures studied from other grasses.

The specialized variety, Erysiphe graminis avenae, as distinguished by Marchal (5) infected Avena fatua, A. orientalis, A. sativa, and Arrhenatherum elatius but not Hordeum vulgare, Secale cereale, or Triticum vulgare. Likewise, he implied that there was no infection on species of Agropyron, Bromus, or Poa.

Salmon (8) reported that conidia from Avena sativa infected A. brevis, A. nuda, A. orientalis, A. sativa, A. sterilis, and A. strigosa, but not Arrhenatherum elatius, Alopecurus pratensis, Dactylis glomerata, Festuca elatior, F. heterophylla, Hordeum vulgare, Lolium italicum, Phleum pratense, Poa annua, Secale cereale, Trisetum pratense, or Triticum vulgare. Conidia from Avena nuda infected A. brevis, A. nuda, and A. sativa. In addition, Salmon (10) states that conidia from Avena sterilis produced full infection on A. sativa, subinfection on A. pratensis and on one leaf of Arrhenatherum avenaceum, but no infection on Bromus sterilis, B. unioloides, Festuca elatior, or Lolium temulentum.

Reed (7) found that conidia from Avena sativa infected Avena barbata Pott, A. brevis Roth, A. chinensis Fisch., A. fatua L., A. fatua L. var. glabrata, A. ludoviciana Dur., A. nuda L., A. nuda L. var. elegantissima, A. orientalis Schreb., A. planiculmis Schrad., A. pratensis L., A. pubescens Huds., A. purpurea Gueldenst, A. sativa L., A. sterilis L., A. strigosa Schreb., A. sulcata F. Gay, and Arrhenatherum avenaceum Beauv.; but there was no infection of Avena bromoides Gouan, A. sempervirens Vill.,

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director. The study was begun by the author at the University of Michigan.

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Holcus lanatus L., Hordeum vulgare L., or Triticum vulgare Vill. Reed (6) reported that oat mildew infected a small percentage of seedlings of Arrhenatherum elatius.

Erysiphe graminis bromi was distinguished by Marchal (5) as occurring on different species of Bromus, notably B. mollis and B. sterilis. Conidia from these two species would not infect Hordeum vulgare. Furthermore, this specialized variety would not infect species of Agropyron or Poa, Avena sativa, Secale cereale, or Triticum vulgare.

Salmon (8, 9) reported results of an extensive study of Erysiphe graminis bromi. His work indicates the occurrence of at least five specialized races, one on each of the following: Bromus arvensis, B. commutatus, B. hordeaceus var. glabrescens, B. interruptus, and B. tectorum.

Marchal (5) found that Erysiphe graminis from Holcus lanatus and Festuca pratensis would not infect Hordeum vulgare, but reactions of other grasses are not given.

Salmon (10) reported that Erysiphe graminis from Dactylis glomerata infected D. glomerata but not Agropyron repens, Avena sativa, Hordeum vulgare, Lolium temulentum, Secale cereale, or Triticum vulgare.

MATERIALS AND METHODS

Selected groups of accessions of 123 species and 8 varieties in 28 genera listed in table 1 were tested with 8 cultures of *Erysiphe graminis* obtained from grasses outside the tribe Hordeae. The methods of handling fungus cultures and grasses and of making inoculations have been fully described (1). The classes of reactions, 0 to 4, used in recording notes were those of Mains and Dietz (4, p. 231).

The term "grass accession" has been explained, as well as the precautions taken to assure accuracy in regard to the specific identity of the grasses used in all experiments (2, p. 65–66).

RESULTS

In table 1 are the general results of inoculating from 1 to many accessions of 123 species and 8 varieties of grasses with 8 cultures of *Erysiphe graminis* from 7 genera. These results demonstrate that powdery mildew is not necessarily restricted to the species of the source genus. The large-scale negative evidence permits a better understanding of the host range of additional isolates of *E. graminis*. The grass accessions listed as infected in table 1 include all cases where definite infection was recorded.

Distinction between resistant and susceptible types of reaction of grass accessions is made in table 2. The 39 accessions of various grass species listed in table 2 were selected because of their significance in illustrating the differences in pathogenicity as well as the extent of the host range of the 8 cultures.

Culture 5. On Avena sativa, from Ann Arbor, Michigan

Of the many grass species tested culture 5 infected only Avena sativa and Trisetum flavescens. Infection of the latter furnishes further proof

TABLE 1.—The number of accessions of various grass species infected after inoculation with 8 cultures of Erysiphe graminis from 7 genera of grasses

	Culture no. and source genus							
	stis	pa 1	z g	nobodhlo	tylis	nus	nca	Koolovia
Species testeda	Agrostis	Avena	Avena	Poly	Dactylis	Bromus	Festuca	Trop
engento de Polición de la Color. La color de Agranda de Color.	23	5	17	8	12	15	16	1
		1	Number	of acc	essions	infect	ed ^b	
egilops crassa Boiss.		0/1	0/1	0/1	0/1	0/2	0/1	••••
. cylindrica Host		0./1	0/1	0/1	0/1	0/1	0/1	••••
. triuncialis L.		0/1						••••
gropyron caninum (L.) Beauv	0/1	0.74	0.74	0.74	0.74	0.74	0.74	ω
. cristatum (L.) Beauv.		0/4	0/4	0/4	0/4	0/4	0/4	0,
. desertorum (Fisch.) Schult.		0/1	0/1	0/1	0/1	0/1	0/1	0
. inerme (Scribn. and Smith) Rdyb		0/12	0/11	0/11	0/11	0/11	0/11	. 0
. intermedium (Host) Beauv.		0/1			************			. 0
. repens (L.) Beauv.		0/1	Ω /1	0./1	0./1	0./1	0./1	0
. semicostatum (Steud.) Nees		0/1	0/1	0/1	0/1	0/1	0/1	0
. sibiricum (Willd.) Beauv.		0/5	0/5	0/5	0/4	0/5	0/5	0
. smithii Rydb.		0/1	0/1	0/1	0/1	0/1	0/1	0
. spicatum (Pursh) Scribn. and	0 /1	0 /19	0 /11	0 /5	0/10	0/19	0/10	
Smith Name of Hook		0/13	0/11	0/5	0/12	0/12	0/12	C
. striatum (Steud.) Nees ex Hook		0 /1	0/1	0/1	0/1	0/1	0/1	••
. subsecundum (Link) Hitchc.		0/1	0/1	0/1	0/1	0/1	0/1	
. trachycaulum (Link) Malte		0/14	0/14	0/14	0/14	0/14	0/14	0
grostis alba L.		0/4	0/4	$\frac{2}{4}$	0/4	0/4	0/4	0
. exarata Trin.		0/1	0/1	1/1	0/1	0/1	0/1	C
. hiemalis (Walt.) B.S.P.		***************************************	0/1	0/1	0/1	0./1	0/1	••
interrupta L.		0 /1	0/1	1/1	0/1	0/1	0/1	•
. palustris Huds.		0/1	0/1	$\frac{1}{1}$	0/1	0/1	0/1	•••
. scabra Willd.			0.71	0/1	0/1	0./1	1/1	
. spica-venti L.			0/1	0/1	0/1	0/1	0/1	••
Longovana acqualia Sabal			0/1	1/1	0/1	0/1	0/1	
lopecurus aequalis Sobol.			0/1	0/1	0/1	0/1	0/1	
. pratensis L.	0/1		0/3	2/3	0/3	0/3	0/3	(
rrhenatherum elatius (L.) Mert. and Koch	0./1	0./1	7 /7	1 /1	0.71	0./1	0.71	
vena brevis Roth		0/1	1/1	1/1	0/1	0/1	0/1	(
. fatua L.			$\frac{1}{1}$	***************************************	0 /1	0./1	0./1	•
. nuda L.		************	$\frac{2/2}{1/1}$		0/1	0/1	0/1	•
. orientalis Schreb.	••••••	•	$\frac{1}{1}$					•
. sativa L.		1/1		0.77	0./1	0 /1	0./1	7
Seckmannia erucaeformis (L.) Host	0/1	0/1	34/34	0/7 $1/1$	0/1	0/1	0/1	(
S. syzigachne (Steud.) Fernald		0/1	0/1		0/1	0/1	0/1	(
romus arvensis L.		$0/1 \\ 0/2$	$0/1 \\ 0/2$	$\frac{1}{1}$	$\frac{0/1}{0/2}$	0/1	$\frac{0}{1}$	• (
B. brizaeformis Fisch, and Mey.		0/2	0/1	0/1	$0/2 \\ 0/1$	$0/2 \\ 0/1$	0/2	•
carinatus Hook, and Arn.			$0/1 \\ 0/2$	$0/1 \\ 0/2$	$0/1 \\ 0/2$		0/1	Č
catharticus Vahl		0/3	0/3	0/2		$\frac{2}{2}$	0/2	C
. commutatus Schrad.		0/1	0/1	$0/3 \\ 0/1$	$0/2 \\ 0/1$	$\frac{1}{3}$ $0/1$	0/3	
3. inermis Leyss.		0/6	0/4	0/6	$0/1 \\ 0/5$		0/1	
3. japonicus Thunb.	*********	0/2	$0/4 \\ 0/2$			$\frac{0}{6}$	0/6	C
3. macrostachys L.	*********	0/2	0/2	$0/2 \\ 0/2$	0/2		0/2	•
3. macrostachys var. lanuginosus Boiss.		0/1			0/2	0/2	0/2	
3. marginatus Nees	********		0/1	0/1	0/1	0/1	0/1	
3. mollis L.		$0/7 \\ 0/2$	0/8	0/8	0/5	3/8	0/8	0
. nexten me management and management of the man	*******		0/2	0/2	0/2	0/2	0/2	
3. polyanthus Scribn		0/1	0/1	0/1	0/1	0/2	0/1	

TABLE 1.—(Continued)

		, J	Culture	no. ai	nd sour	ce gen	us	

	٠ •			Polypogon	60		4.5.	23
	grostis	8	\boldsymbol{a}	oc	Dactylis	Bromus	Festuca	ric
Species tested ^a	7.0	en	en	lyl	cti	om O	st_l	e^{f}
	Ag	Avena	Avena	Po	Da	Br	Fe	Koeleria
	23	5	17	8	12	15	16	19
	20							
		N	umber	of acc	essions	infect	edb	
B. rigidus Roth		0/1	0/1	0/1	0/1	0/1	0/1	
B. rubens L.		0/1	0/1	0/1	0/1	0/1	0/1	•
B. secalinus var. velutinus (Schrad.) Koch		0/1	0/1	0/1	0/1	0/1	0/1	
B. squarrosus L.		0/1	0/1	0/1	0/1	0/1	0/1	
B. tectorum L.		0/1	0/1	0/1	0/1	0/1	0/1	
B. tectorum var. glabratus Spenner		0/1	0/1	0/1	0/1	0/1	0/1	*****
B. vulgaris (Hook.) Shear		0/1	0/1	0/1	0/1	0/1	0/1	
Dactylis glomerata L.		0/8	0/8	0/8	8/8	0/8	0/8	0/
Danthonia parryi Scribn.			0/3	0/1	0/1			
D. semiannularis R. Br.			0/1	0/1	0/1			
		0/2	0/2	$\frac{0}{1}$	0/2	0/2	0/2	0/
Deschampsia caespitosa (L.) Beauv			$0/2 \\ 0/1$	$\frac{1}{1}$	0/1	0/1	1/1	
D. danthonioides (Trin.) Munro D. elongata (Hook.) Munro				0/1			0/1	
		0./5	0/1		0/1	0./4		Δ
Elymus canadensis L.		0/5	0/4	0/5	0/5	0/4	0/5	0,
E. condensatus Presl		0/4	0/4	0/4	0/4	0/4	0/4	0/
E. dahuricus Turcz.		0/1	0/1	0/1	0/1	0/1	0/1	0,
E. glaucus Buckl.		0/4	0/3	0/3	0/2	0/3	0/3	
E. junceus Fisch.		0/2	0/1	0/1	0/1	0/1	0/1	
E. sibiricus L.		0/1	0/1	0/1	0/1	0/1	0/1	
E. triticoides Buckl		0/1	0/1	0/1	0/1	0/1	0/1	0,
E. villosus Muhl.		0/1	0/1	0/1	0/1	0/1	0/1	••••
E. virginicus L.		0/4	0/4	0/4	0/4	0/4	0/4	****
E. virginicus var. glabriflorus (Vasey) Bush	0/1	0/1	0/1	0/1	0/1	0/1	0/1	
E. virginicus var. intermedius (Vasey)								
Bush		0/1	0/1	0/1	0/1	0/1	0/1	,
Festuca arizonica Vasey			0/1	1/1	0/1		0/1	
F. elatior L		0/1	0/1	0/1	0/1	0/1	0/1	
F. elatior var arundinacea (Schreb.)								
Wimm.		0/1	0/1	0/1	0/1	0/1	0/1	
F. gigantea (L.) Vill		0/1	0/1	1/1	0/1	0/1	0/1	
F. idahoensis Elmer			0/1	0/1	0/1	0/1	1/1	0,
F. obtusa Spreng.			0/1	0/1	0/1	0/1	1/1	,
F. occidentalis Hook			0/1	0/1	0/1		1/1	
F. octoflora Walt.		*********	0/1	1/1	1/1	1/1	1/1	****
F. ovina L.			0/1	1/1	0/1	0/1	1/1	
F. rubra L.		0/1	0/2	0/2	0/2	0/1	0/2	
F. rubra var. commutata Gaud.		0/1	0/1	0/1	0/1	0/1	0/1	
F. scabrella Torr.			0/1	0/1	0/1	0/1	0/1	
F. thurberi Vasey			0/1	0/1	0/1	٠, -	1/1	
F. viridula Vasey			0/1	0/1	0/1		1/1	
		0/1	0/1	1/1	0/1	0/1	0/1	
Holous lanatus L.		$0/1 \\ 0/1$	0/1	0/1	0/1	0/1	0/1	
Hordeum bulbosum L.								• •••
H. gussoneanum Parl.		0/1	***************************************	0/1	0/1	0/1		• • • • • • • • • • • • • • • • • • • •
H. jubatum L.		0/1		0/1	0/1			
H. jubatum var. caespitosum (Scribn.)		0.74	0.73	0.41	0.74	0.71		
_ Hitchc.		0/1	0/1	0/1	0/1	0/1		•••
H. murinum L.		0/1	••••••					
H. nodosum L.		0/1			0/1	0/1	0/1	
H. vulgare L.		0/2	0/2	0/2	0/2	0/2	0/2	0
Hystrix patula Moench			0/1	0/1	0/1	0/1	0/1	0

TABLE 1.—(Continued)

Species testeda	Agrostis	g		non				
	•	Avena	Avena	Polypogon	Dactylis	Bromus	Festuca	Koeleria
	23	5	17	8	12	15	16	19
		N	umber	of acc	essions	infect	edb	1
Coeleria cristata (L.) Pers	4/5	0/5	0/7	6/7	0/7	0/5	0/7	6/6
olium multiflorum Lam.		0/1	0/1	0/1	0/1	0/1	0/1	0/1
. perenne L.		0/1	0/1	0/1	0/1	0/1	0/1	0/1
Iilium effusum L.		0/1	0/1	1/1	0/1		0/1	
halaris arundinacea L.		0/1	0/1	0/1	0/1	0/1	0/1	0/:
hleum pratense L.		0/3	0/3	3/3	0/3	0/2	0/3	0/3
oa ampla Merr.		0/7	0/31	0/3	0/3	0/3	0/30	0/7
oa arachnifera Torr.			0/1	0/1	0/1	0/1	0/1	-, ,
. arctica R. Br.		0/1			-, -	-7 -		******
arida Vasey			0/1				0/1	
'. bulbosa L.		0/1	0/4				0/4	0/:
			- '		0./4	0/4	0/9	0/
canbyi (Scribn.) Piper		0/3	0/8	0/4	0/4	•		
compressa L.		0/3	0/3	1/1		0 /1	0/3	0/
curta Rydb.		0/1	0/1	1/1		0/1	0/1	0/
. cusickii Vasey		0/1	0/2	1/1	0./1	0/1	0/2	0/
epilis Scribn.		0/1	0/2	0/1	0/1	0/1	0/2	0/
. gracillima Vasey		0/1	0/3	0/2	0/1	0/2	0/3	0/
. interior Rydb.			0/1	0/1	0/1	0/1	0/1	
. juncifolia Scribn.		0/2	0/5	0/1	0/1	0/1	0/5	0/
. nemoralis L.		0/3	0/8	0/5	0/3	0/5	0/4	0/
. nervosa (Hook.) Vasey			0/2	0/1	0/1	0/1	0/2	
. nevadensis Vasey	. 0/1	0/3	0/6				0/6	0/
P. palustris L.	0/2	0/3	0/6	0/3	0/3	0/3	0/6	0/
P. pratensis L.	. 0/4	0/14	0/15	1/7		0/6	0/5	0/1
c. scabrella (Thurb.) Benth.		0/1	0/3	0/3	0/3	0/3	0/3	0/
, secunda Presl			0/6	0/3	0/4	0/3	0/6	0/
sphondyloides Trin.		0/3	0/5	0/1	0/1	0/1	0/4	0/
sterilis Bieb.		***********	0/1	0/1	0/1	0/1	0/1	0/
Polypogon monspeliensis (L.) Desf	0/1		0/1	$1/\overline{1}$	0/1	0/1	0/1	0/
Puccinellia distans (L.) Parl.			0/1	1/1	1/1	0/1	0/1	0/
ecale cereale L.	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/
itanion hystrix (Nutt.) J. G. Smith		0/1	0/1	0/1	0/1	0/1	0/1	0/
. jubatum J. G. Smith		0/1	0/1	0/1	0/1	0/1	$0/1 \\ 0/1$	0/
risetum flavescens (L.) Beauv.	0/1	1/1	0/1	1/1	,			
spicatum (L.) Richt.	0/1				0./1		0/1	•••••
riticum aestivum L.		0/1	$0/1 \\ 0/1$	$0/1 \\ 0/1$	$0/1 \\ 0/1$	0/1	$\frac{1}{1}$	0/

^a Hitchcock (3) was followed wherever possible for the nomenclature of the grasses.

^b Numerator of fraction refers to number of accessions infected; denominator refers to total number of accessions tested.

that Avena mildews are not necessarily restricted to species of Avena. The many negative results give a more complete understanding of the host range.

Culture 17. On Avena sativa, from Pullman, Washington

Culture 17, started from dry, infected leaves sent by Dr. George W. Fischer, infected Avena brevis, A. fatua, A. nuda, and A. orientalis. On varieties of A. sativa the following reactions were recorded: very susceptible

TABLE 2.—Reactions of 39 accessions of grasses to 8 cultures of Erysiphe graminis

Types of reaction Agrostis exarata 21			Cult	are n	o. aı	d sour	ce gen	us	
Agrostis exarata 21									
Agrostis exarata 21			nc						
Agrostis exarata 21		tis	· BC			lis	83	ä	'n.
Agrostis exarata 21	Grass accessions	SO	ď	ua	ua	ty	m	tac	le
Agrostis exarata 21		g	ijo,	v_c	ve	ac	3.0	es	Zoe
Types of reaction Agrostis exarata 21		A	P	4	A	D	B	Ħ	B
Agrostis exarata 21		23	8	5	17	12	15	16	19
Agrostis exarata 21				<i>m</i>					
Agrostis palustris 22				Тур	es 01	react	10nb		
Alopecurus pratensis 234				0	_	0	0		0
Arrhenatherum elatius 23 0 3-4 0 1 0 0 0 Avena sativa 369 0 4 4 0 0 0 0 Beckmannia erucaeformis 11 0 2 0 0 0 0 0 Bromus carinatus 229 0 0 0 0 0 0 0 0 0 </td <td></td> <td></td> <td>2-3</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td>			2-3	0	0	0	0	0	0
Avena sativa 369	Alopecurus pratensis 234	. 0	3-4	0	0.0	0	0		
Beckmannia erucaeformis 11			3-4	0	1	0	0	0	0
Beckmannia syzigachne 12			0	4	4	0	0	0	0
Bromus carinatus 229	Beckmannia erucaeformis 11	. 0	2	0	0.	0	0	0	0
Dactylis glomerata 31	Beckmannia syzigachne 12	. 0-3-	3-4	0	0	0	0	0	0
Deschampsia caespitosa 9	Bromus carinatus 229	0	0	0	0	0	4	0	0
Deschampsia caespitosa 10	Dactylis glomerata 31	. 0	0	0	0	3-4	0	0	0
Deschampsia danthonioides 318	Deschampsia caespitosa 9	. 2+	3	0	0	0	0	0	0
Festuca idahoensis 250	Deschampsia caespitosa 10	. 3 –	2	0	0	0	0	0	0
Festuca octoflora 323	Deschampsia danthonioides 318		3 -	0	0	0	1	3-4	
Festuca rubra var. commutata 85	Festuca idahoensis 250		0	0	0	0	0	4	0
Festuca rubra var. commutata 85	Festuca octoflora 323	. 4	2 -	0	0	3	0-1	4	
Holcus lanatus 187 2-3 0 0 0 0 Koeleria cristata 32 2 1-2 0 0 0 0 3 Koeleria cristata 34 0 1 0 0 0 0 4 Koeleria cristata 35 1 2-3 0 0 0 0 4 Milium effusum 223 2-3 0 <td></td> <td></td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td></td>			0	0	0	0	0	0	
Koeleria cristata 32 2 1-2 0 0 0 0 3 Koeleria cristata 33 2 1+ 0 0 0 0 0 2 Koeleria cristata 34 0 1 0			2-3	0	0	0	0	0	
Koeleria cristata 34 0 1 0 0 0 0 4 Koeleria cristata 35 1 2-3 0 0 0 0 0 4 Millium effusum 223 2-3 0	Koeleria cristata 32	2	1-2	0	0	0	0	0	3
Xeoleria cristata 35	Koeleria cristata 33	2	1 +	0	0	0	0 :	0	2
Xeoleria cristata 35	Koeleria cristata 34	0	1	0	0	0	0	0	4
Milium effusum 223 2-3 0 0 0 0 Phlataris arundinacea 13 0 0 0 0 0 0 0 0 Phleum pratense 15 0 2-3 0 0 0 0 0 0 Poa canbyi 43 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			2-3	0	0	0	0	Õ	4
Phalaris arundinacea 13 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0						-	-		
Phleum pratense 15 0 2-3 0 0 0 0 Phleum pratense 16 2-3 1-3 0 0 0 0 Poa canbyi 43 0 0 0 0 0 0 Poa compressa 49 0 0 0 0 0 0 Poa compressa 50 0 2+ 0 0 0 0 0 Poa curta 51 2- 0			0		0	0	0	0	0
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$			-	0		-	-	-	. 0
Poa canbyi 43 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0				0			·		0
Poa compressa 49 0				0	-			0	ŏ
Poa compressa 50 0 2+ 0					-				0
Poa curta 51 2- 0 0 0 0 0 0 Poa nevadensis 59 0 0 0 0 0 0 0 Poa palustris 57 0 0 0 0 0 0 0 Poa palustris 65 0 0 0 0 0 0 0 0 Poa pratensis 68 0 0-1 0 0 0 0 0 0 0 Poa pratensis 70 0 0 0 0 0 0 0 0 0 0 Poa pratensis 70 0 0 0 0 0 0 0 0 0 0 0 0 Polypogon monspeliensis 230 0 4 0 0 0 0 0 0 Puccinellia distans 251 0 2- 0 0 2+ 0 0 0 Trisetum flavescens 228 0 2-3 3 0 0 0 0					-	_			ō
Poa nevadensis 59 0 0 0 0 0 Poa palustris 57 0 0 0 0 0 Poa palustris 65 0 0 0 0 0 Poa pratensis 68 0 0-1 0 0 0 0 Poa pratensis 69 0 0 0 0 0 0 Poa pratensis 70 0 0 0 0 0 0 Polypogon monspeliensis 230 0 4 0 0 0 0 Puccinellia distans 251 0 2- 0 2+ 0 0 Trisetum flavescens 228 0 2-3 3 0 0 0							_		0
Poa palustris 57 0 0 0 0 0 Poa palustris 65 0 0 0 0 0 Poa pratensis 68 0 0-1 0 0 0 0 Poa pratensis 69 0 0 0 0 0 0 0 Poa pratensis 70 0 0 0 0 0 0 0 Polypogon monspeliensis 230 0 4 0 0 0 0 0 Puccinellia distans 251 0 2- 0 2+ 0 0 0 Trisetum flavescens 228 0 2-3 3 0 0 0 0			-	-		-	-		0
Poa palustris 65 0 0 0 0 0 Poa pratensis 68 0 0-1 0 0 0 0 Poa pratensis 69 0 0 0 0 0 0 0 Poa pratensis 70 0 0 0 0 0 0 0 0 Polypogon monspeliensis 230 0 4 0 0 0 0 0 0 0 Puccinellia distans 251 0 2- 0 2+ 0					-				ŏ
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Poa nalustris 65	ň		-					ŏ
Poa pratensis 69 0									Ö
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$						-			0
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$							100		0
$egin{array}{cccccccccccccccccccccccccccccccccccc$	Polymogon moneneliencie 920	0	-	-	-				. 0
Trisetum flavescens 228 0 2-3 3 0 0 0	Pugginglia dietane 951	0						-	Ö
2.10010110).001000110 220			_				0 1		U
1718cium spicatum 549 2+						-		-	
	Trisetum spicatum 329	0	0	0	0	0	*******	2+	

a Accession numbers assigned to grass collections by the writer.

b Classes of reaction expressed according to the system of Mains and Dietz (4): 0—Highly resistant. Little or no mycelium. Chlorotic or necrotic flecks on

-Highly resistant. Little or no mycelium. Chlorotic or necrotic flecks on some hosts.

1—Very resistant. Slight to moderate mycelium. No sporulation. Chlorotic or necrotic spots may develop.

2—Moderately resistant. Moderate to abundant mycelium. Slight sporulation. Chlorotic or necrotic areas may develop.

3—Moderately susceptible. Moderate to abundant mycelium. Moderate sporulation.

4-Very susceptible. Abundant mycelium and sporulation.

.. - Indicates no test was made.

—Markton C.I. 2053, Richland C.I. 787, Boone C.I. 3305, Marion C.I. 3247, Bond C.I. 2733, Victoria C.I. 2401, Anthony C.I. 2143, Columbia C.I. 2820, D-67, C.I. 2870, Flughafer C.I. 3516, Fulghum C.I. 708, Gopher C.I. 2027, Green Mt. C.I. 1892, Hancock C.I. 3346, Iomine C.I. 2827, Makota C.I. 2885, Rainbow C.I. 2345, Ruakura C.I. 2025, Schwartzhafer C.I. 3520, White Tartar C.I. 551, Sterisel C.I. 2891, Victoria C.I. 1197 and White Tartar C.I. 3139; moderately susceptible—Iogold C.I. 2329, Erban C.I. 3477, Glabrota C.I. 2630. Landhafer C.I. 3522, Mutica Ukraina C.I. 3259, Richland C.I. 847, Fulton C.I. 3327, Bannock C.I. 2592, Red Rustproof C.I. 458, Marida C.I. 2571 and Joanette C.I. 2331.

A very resistant reaction on Arrhenatherum elatius was the only infection outside the genus Avena, and only 3 of 81 plants tested of this grass gave this reaction. The discrepancies between the results with Avena mildew obtained by previous workers in regard to the infection or non-infection of A. elatius could easily be attributed to the variability in A. elatius as well as to probable differences between the oat mildew cultures studied by each of the workers. A. elatius was also variable in its reaction to culture 8 from Polypogon monspeliensis. No variety of Avena sativa was resistant in the present study. Trisetum flavescens was uniformly, moderately susceptible to culture 5 and highly resistant to culture 17. On the basis of the reactions of this grass species to these two cultures pathogenic specialization is demonstrated in Erysiphe graminis from Avena.

Culture 12. On Dactylis glomerata, from Ann Arbor, Michigan

Culture 12 produced different types of infection on several accessions of Dactylis glomerata and moderately resistant reactions in Festuca octoflora and Puccinellia distans. The differences in reaction among accessions of Dactylis glomerata afford an opportunity to select for mildew resistance. This mildew culture probably represents a distinct specialized variety of Erysiphe graminis.

Culture 15. On Bromus carinatus, from Pullman, Washington

Culture 15 was started from infected plants sent by Dr. George W. Fischer and infected only a few species of *Bromus*. The very resistant reaction of *Festuca octoflora* furnishes the only evidence of infection outside the genus *Bromus*. Chlorotic spots were produced on *Deschampsia danthonioides* indicating that other susceptible grasses might eventually be found. The results in table 1 provide considerable negative evidence that has been lacking in all previously published records.

Culture 16. On Festuca idahoensis, from Pullman, Washington

Culture 16 was obtained from infected plants sent by Dr. George W. Fischer. It infected Agrostis scabra, Deschampsia danthonioides, various species of Festuca, and Trisetum spicatum. The species of Festuca varied in reaction from very susceptible to highly resistant. Certain species of Festuca were not infected by culture 16 but were susceptible to other cul-

tures which originated on other genera, viz., culture 8 from Polypogon and cultures from Poa previously reported (2).

Culture 19. On Koeleria cristata, from Pullman, Washington

Culture 19 was collected in the Soil Conservation Service nurseries. It infected only collections of *Koeleria cristata*, and these differed in reaction. Three were very susceptible, two were moderately susceptible, and one was moderately resistant. Festuca octoflora and Deschampsia danthonioides were critical species to demonstrate infection outside the source genus for the cultures from Bromus, Dactylis, and Festuca. Unfortunately these two grasses were not available at the time culture 19 was studied.

Culture 8. On Polypogon monspeliensis, from Yakima, Washington

Culture 8 produced infection on many grass species of the genera in several tribes as follows: Tribe Festuceae—Festuca, Poa, and Puccinellia; Tribe Aveneae—Arrhenatherum, Deschampsia, Holcus, Koeleria, and Trisetum; Tribe Agrostideae—Agrostis, Alopecurus, Milium, Phleum, and Polypogon; and Tribe Chlorideae—Beckmannia. Culture 8 stemmed from a single pustule transfer, and a single colony isolation from this culture did not differ in host range. Therefore, culture 8 can be regarded as an individual race and not a mixture.

Culture 23. On Agrostis exarata, from Klickitat County, Washington

Culture 23 was started from infected, living plants sent by Dr. George W. Fischer. Infection was produced on Agrostis alba, A. exarata, Beckmannia syzigachne, Deschampsia caespitosa, Festuca octoflora, and Koeleria cristata. The infection of grasses in several tribes is somewhat similar to that produced by culture 8 from Polypogon. The two cultures are, of course, very different. Results with these two cultures and others suggest that there is probably a complex group of mildew races with overlapping host ranges affecting the grasses, especially Agrostis, Phleum, Beckmannia, and Polypogon in the tribe Agrostideae. Powdery mildew is commonly reported on these genera in the Pacific Northwest.

DISCUSSION

The various cultures differ in degree of specialization. Culture 8 from Polypogon monspeliensis infected 26 species of 17 genera in 4 tribes, and culture 23 from Agrostis exarata infected 7 species of 6 genera in 4 tribes of the Gramineae. Erysiphe graminis from Avena, Bromus, Dactylis, and Festuca have so far produced infection on a very few species outside the genus from which they were collected. Culture 19 from Koeleria infected only Koeleria cristata.

Certain cultures are comparable to previously described specialized varieties. Cultures 5 and 17 could be classed as *Erysiphe graminis avenae* and culture 15 as *E. graminis bromi*. If this system were continued mildew

cultures would be given varietal names based on the host genus from which they were collected. Since it was demonstrated previously (1, 2) and in the present study that most mildew cultures studied will infect species of two or more genera, two or several varietal names might be given to the same mildew race. Culture 12 from Dactylis glomerata could be named for either Dactylis or Festuca, and culture 16 from Festuca idahoensis might be named for Festuca or Deschampsia, depending upon the host genus from which the mildew races were obtained. Culture 8 from Polypogon monspeliensis and culture 23 from Agrostis exarata have wide host ranges. Grasses in several genera were distinctly susceptible to these two cultures, and thus several varietal names based on the source genus might be given to these two cultures, dependent again on the host genus on which these mildews are collected.

Many of these cultures, especially cultures 8 and 23, probably could be collected again on grass species of different host genera. Indiscriminate application of varietal names to races of *Erysiphe graminis* based only on the source genus can lead to repeated duplications, unavoidable synonyms, and unnecessary confusion. It would appear more desirable, at least for the present, to use laboratory or numerical designations for cultures of the fungus.

It is significant that all cultures of *Erysiphe graminis* from grasses outside the tribe Hordeae studied in the present investigation and previously reported from the genus *Poa* (2) infected only grasses outside this tribe. It was reported previously (1) that cultures studied from grasses in the tribe Hordeae infected only grasses in that tribe.

With the information now at hand there is little support for a concept of specialization of races of *E. graminis* to any given grass genus. In only a few cases was this noted, as follows: culture 20 from *Poa palustris*, previously reported (2), and culture 19 from *Koeleria cristata*. On the evidence of infection of species outside the source genus, obtained from the other cultures, it appears that even with cultures 19 and 20 other genera might have been infected if more collections and species had been tested.

Instead of narrow host specialization with restriction of infection of any mildew race to species of one genus as was believed and taught for over 40 years, since specialized varieties were distinguished by Marchal (5) in 1902, the situation regarding specialization of pathogenicity in *Erysiphe graminis* is quite the contrary for the most part. It has been demonstrated previously (1, 2) and in the present paper, that the majority of the races of *E. graminis* studied by the writer will infect species of two or more genera. With some cultures the host range is extensive, including many species and several genera.

Certain grasses are susceptible to several very different mildew races. The overlapping host ranges of different mildew races is such that powdery mildew occurring on some grass species might be any one race or a mixture of several mildew races. The pathogenicity of mildew races occurring on

grasses in the tribe Hordeae has been previously described (1). study mildew cultures that infected barley were isolated from Agropyron repens and Elymus dahuricus. Another mildew culture that infected wheat was isolated from E. dahuricus. A mildew culture that infected barley and a few wild grasses and an Agropyron mildew that infected many wild grasses but not rye, wheat, or barley were found together as a mixture on Another mixture on E. dahuricus contained a race that infected wheat and several wild grasses and a race that infected wild grasses, principally species of Agropyron and Elymus, but not wheat, barley, or rye. With this information we are better able to understand the origin of mildew outbreaks, particularly with reference to the sources of inoculum. grasses can harbor wheat and barley mildews and supply the inoculum for infection of these cereals. Likewise mildew-infected wheat and barley companion crops with grasses, or fields of these cereals near grass stands, can be the sources of infection of grasses, especially species of Agropyron and A probable illustration of the latter was observed by the writer during the early summer of 1940 in the Soil Conservation Service nurseries at Pullman, Washington. A heavily mildew-infected cover crop of wheat was adjacent to a grass observational row nursery containing many collections of species of Agropyron with very little infection. After about one week of wet weather a heavy outbreak of powdery mildew developed on many of the Agropyron species. The rapidity of the outbreak suggested a source of abundant inoculum, and the slight amount of infection already on the Agropyron species appeared to be an inadequate source. pointed to mildew inoculum from the adjacent wheat cover crop.

Likewise, with accumulating information about the pathogenicity of other races of *Erysiphe graminis*, it will be easier to understand certain phases in the etiology of the disease on other grasses. A mildew outbreak occurred in western Oregon during 1941 on Chewings fescue, *Festuca rubra* var. *commutata*. Although a culture was not studied, there are certain facts that suggest mildew on *Poa pratensis* as a source of the outbreak. Mildew on *P. pratensis* is very common in the Pacific Northwest. One accession of Chewings fescue studied by the writer was very susceptible to mildew from *Poa pratensis* (2), but was resistant to mildew from *Festuca idahoensis*.

Another example was an outbreak of mildew on *Phalaris arundinacea* near the college golf course at Pullman, Washington, reported to the writer by Dr. George W. Fischer. *P. arundinacea* is seldom reported infected by mildew. When informed that the writer had produced infection on *P. arundinacea* with mildew from *Poa pratensis*, Dr. Fischer replied that this could very possibly have been the source of the outbreak, since *P. pratensis* was the only other grass infected with mildew within a considerable distance of the area.

Although the relationships in these examples are based largely on field observations, they are supported by experimental evidence. The writer believes that these examples offer plausible explanations of mildew outbreaks

on certain grasses, and with the accumulating information about the host ranges of additional mildew races, such reasoning should aid in understanding certain phases in the epiphytology of the disease.

While nothing conclusive has been reported concerning the sexual nature of Erysiphe graminis and although hybridization between races is a theoretical suggestion, the fact that so much variation is found in this species is ample evidence of the genetic segregations which have already taken place. There are opportunities for hybridization between widely different races. since certain grass species are susceptible to a number of races. In a study of the mildew races attacking grasses in the tribe Hordeae (1) it was demonstrated that mildews which infect barley, wheat, Agropyron, and Elymus can all occur together on the same grass, especially on certain species of Agropyron and Elymus. The situation with other mildew races is comparable. For example, Festuca octoflora is susceptible to mildew races from Agrostis, Dactylis, Festuca, and Poa and to a lesser extent also to races from Polypogon and Bromus. Koeleria cristata is susceptible to mildew races from Poa, Koeleria, Polypogon, and to a lesser extent also from Agrostis. Many other examples could be given in which grasses are susceptible to two or more mildew races. All of these grasses are potential sites for hybridization between races that may be widely different pathogenically. Considering these possibilities it is not surprising to find great variation for pathogenicity among races in Erysiphe graminis.

Results on specalization suggest that there have been genetic segregations in *Erysiphe graminis* that have established themselves on certain grass species. In studies of pathogenic specialization probably only a few of these segregates have been studied. That some appear to be narrowly specialized and others less so is important, but viewing *E. graminis* as a broad species comprising many genetic combinations and constantly producing new segregates, we may anticipate descriptions of additional new races that cannot be readily classified on the basis of a varietal system of classification.

SUMMARY

Selected groups of 360 accessions of 123 species and 8 varieties of grasses in 28 genera were inoculated with 8 cultures of *Erysiphe graminis* from grasses outside the tribe Hordeae as follows: 2 cultures from *Avena* and one culture each from *Agrostis*, *Bromus*, *Dactylis*, *Festuca*, *Koeleria*, and *Polypogon*.

Seven of the cultures infected grass species outside the genus from which they were collected. Powdery mildew from *Koeleria cristata* infected only this species.

Two cultures from Avena sativa were different pathogenically, and pathogenic specialization is therefore demonstrated in Erysiphe graminis from Avena. No variety of A. sativa was resistant in the present study.

Many grass species are susceptible to two or more widely different mildew races and thus perhaps may present the opportunity for hybridization between races.

Certain races may perhaps bear varietal names to advantage if they continue to have distinctly narrow specialization. Several mildew cultures have wide host ranges, and a varietal designation for such races appears to be impracticable.

Variation in mildew reaction between collections of most grass species and between individual plants in many collections affords ample opportunity to select for mildew resistance.

Erusiphe graminis occurring on a given grass species can no longer be automatically assumed to be a specialized variety restricted in infection to species of the source genus. On the contrary, inoculation results demonstrate that it may be any one of a number of mildew races or a mixture of races.

The evidence of a general lack of specialization of mildew races to any given genus permits recognition of the possibilities of hybridization between races, the possible sources of inoculum in the initiation of mildew infections, and explanations of certain phases of the etiology and of the epiphytology of the disease.

KENTUCKY AGRICULTURAL EXERIMENT STATION, LEXINGTON, KENTUCKY.

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THE SEASONAL DEVELOPMENT AND THE DEFOLIATING EFFECT OF CRONARTIUM RIBICOLA ON NATURALLY INFECTED RIBES ROEZLI AND R. NEVADENSE

JAMES W. KIMMEY1,2

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INTRODUCTION

Ribes roezli and R. nevadense are the two principal ribes species associated with sugar pine in the California sugar pine region. Since this region differs not only in host species but also in weather characteristics from other parts of North America where experience with white pine blister rust has been gained, the development of the rust on these host plants and their responses in the existing environment are of particular interest and concern in the rust control problem here.

The relative susceptibility of these two species was studied earlier on bushes transplanted into British Columbia (6). Ribes nevadense was rather susceptible in comparison with other western ribes species (4) and produced many telia, while R. roezli was extremely susceptible but shed its infected leaves early. Therefore, it produced few telia. With the appearance of rust in California, it was possible in 1938, 1941, and 1943 near Montgomery Creek and Hatchet Creek to observe the development of the rust on these species in their native habitat, and to correlate this with the danger to neighboring sugar pines. Some critical details are herein reported.

METHODS AND BASES

Representative infected plants of *Ribes roezli* and *R. nevadense*, varying in degree of initial infection, and growing under varying site moisture conditions and exposure to direct sunlight, were selected for observation. On extremely large plants detailed data were taken only on one or two large representative branches rather than on the entire plants.

Detailed data on host and rust development and reaction were taken separately for each plant or each branch selected. Biweekly examinations were made from July, when nearly all leaves were formed and the rust was becoming well established, to late October, when nearly all leaves had dropped.

In 1938 a total of 100 plants of Ribes roezli and 8 of R. nevadense scattered over both Montgomery Creek and Hatchet Creek drainages were

¹ Associate Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

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The ribes that became infected with Cronartium ribicola that spring were infected by aeciospores blown from distant northern pine infections. During the year aeciospores of the pinyon rust, Cronartium occidentale, apparently were blown into this locality from the south. Only 43 of the 100 selected plants of R. roezli were infected with C. ribicola, as determined by differential staining of telial columns (1, 2), the most satisfactory method known for differentiating between the two rusts on ribes. By 1941 and in 1943 there were aeciospore-producing sugar pine infections on the study areas. In 1941 rust samples from each of the 65 plants of R. roezli and 10 of R. nevadense selected on the Montgomery Creek area were tested microchemically and determined as C. ribicola. The 62 plants of R. roezli and the 18 of R. nevadense selected in 1943 on the Hatchet Creek area were scattered about two infected sugar pines bearing abundant aecial cankers, so it was not considered necessary to test rust samples from each plant. All samples tested from 20 plants were C. ribicola. No C. occidentale was found in these localities in 1941 or 1943.

The amount of live-stem (living branchwood) expressed in linear feet is a measure commonly used by blister rust workers for ribes. The data herein are presented on this same unit basis.

RESULTS

The weather in 1943 was similar to that in 1938 and there was great similarity in the rust development on ribes and its effect on ribes defoliation in these two years. Because of the similarity, many of the data of 1938 are omitted in tables and figures. However, the general results and conclusions herein reported are based upon the observations in 1938 as well as those in 1941 and 1943.

Weather

Spring weather favored infection of ribes from aeciospores in each of the three test years. This was not the case in some of the intervening years, particularly in 1942. Although the initial ribes infection in 1938 was not heavy, late spring rains favored intensification of the rust. There were no summer rains in 1938, and there was no rain from June 21 to October 11 in 1943, but the summer of 1941 was abnormally cool with comparatively frequent rains (Table 1).

TABLE 1.—The distribution of rainfall in Montgomery Creek and Hatchet Creek areas in California from July through October in 1938, 1941, and 1943

\mathbf{Month}	Dates	s on which rainfall	occurred
	1938	1941	1943
July August September	27–28	$\begin{array}{c} 15-16-17 \\ 15-16-29-30 \\ 1-2-12-18-19 \end{array}$	
October	1-15-16-23- 28-29-30	10-12-19-20- 26-27-28	11-17-18-19-20- 21-22-23-24

Ribes Defoliation

The very wet May of 1941 provided good conditions for initial ribes infection from pines, and the abnormally frequent summer rains intensified the rust infection on the ribes leaves. On Ribes roezli the intensity of the rust infection caused considerable premature leaf-fall. This early dropping of leaves and the abundance of moisture stimulated the formation of new leaves throughout the season on this species. The average extent of new leaf formation and the average leaf-fall for infected and uninfected leaves of this species throughout the 1941 season are in table 2. The plants have been divided into light forms: the open-form plants are those that received very little or no shade; the part-shade-form plants received shade and direct sunlight in about equal proportions; and the shade-form plants received little or no direct sunlight. The open-form plants bore the most leaves per hundred feet of live stem and the shade-form plants the least. The fewer leaves on the part-shade and shade-form plants were largely compensated for by their larger size, making leaf area per hundred feet of live stem nearly equal on all forms. Premature leaf-fall was greatest on the open-form plants and least on the shade-form plants. Precocious leaf-fall induced by the rust infection is generally more severe on gooseberries than on currants (5, p. 42). On Ribes nevadense premature leaf-fall was not great and the formation of new leaves during the summer was negligible (Table 2). Open-form plants of R. nevadense were practially nonexistent on the study areas, because cattle readily browse on this smooth-stemmed species wherever the plants are not protected or screened by other vegetation. Observations on the open-form plants of this species have not been included.

Table 3 shows the average leaf-fall in 1943 when there were no summer rains. The last spring rain, on June 21, caused the only pronounced wave of rust intensification on Ribes roezli. Rust development is considerably slower on R. nevadense than on R. roezli and by June 21 the uredia on R. nevadense were not developed sufficiently to cause heavy intensification of the infection. The part-shade and shade-form plants of R. roezli did not produce the amount of intensification from the June 21 rain that the openform plants did (Table 3). This was due to the fact that the development of the uredia on these shaded plants was not so advanced as that on the open-form plants by June 21. Table 3 also shows how this heavier intensification of the rust on the open-form plants caused much greater premature leaf-fall than occurred on the shaded plants. The premature leaf-fall of R. roezli, in general, was not so great in 1943 as in 1941 because there were no summer rains to intensify the infection. However, the lack of summer rains probably hastened the normal dropping of uninfected leaves, and on most plants the infected leaves were shed before the uninfected leaves. When soil moisture for individual plants is considered, the number of new leaves formed on R. roezli during the summer of 1943 fairly well depicts the severity of the premature leaf-fall. The formation of the new leaves on *R. nevadense* in 1943 was largely stimulated by the premature leaf-fall caused by insects and anthracnose rather than by blister rust infection.

TABLE 2.—Average progressive leaf-fall per hundred feet of live stem for both infected and uninfected leaves for each light form of Ribes roezli and R. nevadense at the Montgomery Creek Area in 1941

			of leaves t of live s		Numbe		ted leaves j ive stem	per 100
ligh and	ecies, t form, l date served	Total dropped	Formed since previous exam.	Total attached	Total dropped	Remaining from previ- ous exam.	Infected since previous exam.	Total attached
Ribes roezl Open	i July 26-29 Aug. 12-14 Aug. 25-27 Sept. 9-10 Sept. 23-24 Oct. 7-8 Oct. 23	352 1583 2280 2754 3013 3196 3243	113 63 61 14 9 5	2687 1570 918 505 258 84 42	201 1352 2040 2462 2656 2786 2814	365 450 237 139 30 10	1030 233 99 21 9	1246 1125 658 334 160 39
Part shade	July 26-29 Aug. 12-14 Aug. 25-27 Sept. 9-10 Sept. 23-24 Oct. 7-8 Oct. 23	243 948 1677 2072 2283 2541 2636	48 20 23 16 1	2303 1646 937 566 370 113 18	176 869 1563 1931 2097 2279 2334	603 653 330 212 51 4	745 116 49 18 10	1223 1275 697 378 230 58 4
Shade	July 26-29 Aug. 12-14 Aug. 25-27 Sept. 9-10 Sept. 23-24 Oct. 7-8 Oct. 23	217 805 1301 1616 1842 2127 2300	48 29 28 10 1	2018 1479 1004 717 502 218 45	150 735 1231 1535 1731 1965 2078	416 649 420 291 84 16	762 75 67 20 51	974 1151 724 488 312 129 16
R. nevader Part shade	July 26-29 Aug. 12-14 Aug. 25-27 Sept. 9-10 Sept. 23-24 Oct. 7-8 Oct. 23	16 147 329 640 871 1325 1689	0 0 0 0 0 0 0 2	1704 1573 1390 1079 848 394 32	5 63 245 556 787 1241 1602	320 1208 1012 786 362 16	1070 114 5 30 15	378 1390 1323 1017 815 377 16
Shade	July 26–29 Aug. 12–14 Aug. 25–27 Sept. 9–10 Sept. 23–24 Oct. 7–8 Oct. 23	51 141 243 436 882 1428 1919	0 1 0 0 0 0	1979 1889 1788 1595 1150 603 112	2 84 186 379 825 1273 1593	764 968 938 591 328 63	306 163 99 183 56 0	846 1071 1131 1037 775 383 63

The first frosts in the autumn appeared to affect the fall of infected leaves of *Ribes roezli* more than uninfected leaves. Later frosts were so severe that both uninfected and infected leaves dropped. There appeared to be little difference in effect of frost upon the leaf shedding of *R. nevadense*

whether infected or not. Light early frosts caused the dropping of few leaves of this species and later heavy frosts caused many leaves to fall.

TABLE 3.—Average progressive leaf-fall per hundred feet of live stem for both infected and uninfected leaves for each light form of Ribes roezli and R. nevadense at the Hatchet Creek Area in 1943

			r of leave et of live s		Numbe	r of infect feet of li		per 100
ligh an	pecies, t form, d date served	Total dropped	Formed since previous exam.	Total attached	Total dropped	Remaining from previ- ous exam.	Infected since previ- ous exam.	Total attached
Ribes roezl	li							
Open	July 16-19 July 28-31 Aug. 13-16 Aug. 27-28 Sept. 10-11 Sept. 24-25 Oct. 9 Oct. 24	10 617 1724 2273 2819 3019 3109 3252	28 12 11 23 13 11	3395 2785 1690 1141 618 431 352 210	10 614 1705 2254 2750 2907 2973 3016	1506 696 679 296 139 73 30	280 541 113 0 0 0	2130 1787 1236 792 296 139 73 30
Part shade	July 16-19 July 28-30 Aug. 13-16 Aug. 27-28 Sept. 10-11 Sept. 24-25 Oct. 9 Oct. 24	47 371 820 1258 1636 2042 2353 2775	11 5 2 0 1 0	2859 2545 2102 1666 1288 883 572 150	47 371 718 1117 1422 1564 1654 1728	1016 765 575 315 173 83 9	95 208 45 0 0	1340 1111 973 620 315 173 83
Shade	July 17-19 July 29-31 Aug. 13-16 Aug. 27-28 Sept. 10-11 Sept. 24-25 Oct. 9 Oct. 24	32 74 218 404 773 1265 1662 2217	22 10 1 1 1 1 0	2445 2425 2291 2106 1737 1261 866 311	30 72 204 390 731 878 963 991	592 476 591 271 126 40 12	16 300 21 0 0	635 608 776 612 271 126 40 12
R. nevaden	ise		- L					
Part shade	July 16-19 July 28-31 Aug. 13-16 Aug. 27-28 Sept. 10-11 Sept. 24-25 Oct. 9 Oct. 24	2 145 248 378 473 629 804 1089	8 1 1 2 2 0 0	1495 1361 1261 1138 1045 892 717 432	1 97 152 186 234 294 352 427	262 266 323 313 253 196 121	59 89 38 0 0	358 321 355 361 313 253 196 121
Shade	July 16-19 July 29-31 Aug. 13-16 Aug. 27-28 Sept. 10-11 Sept. 24-25 Oct. 9 Oct. 24	2 43 48 66 94 217 397 509	5 2 0 0 0 0	1149 1113 1110 1091 1064 942 762 649	2 10 15 33 61 160 259 294	332 331 383 364 265 166 131	4 71 8 0 0 0	340 336 402 392 364 265 166 131

Beside frosts, severity of infection, and degree of exposure of the plants to direct sunlight, two other factors influenced premature leaf-fall of infected *Ribes roezli*. One of these was the soil moisture of the site. The more moist

the site the more retentive were the infected leaves when the severity of infection was the same. However, on wet sites the infection usually was more severe, because the leaves were more succulent and therefore more susceptible to attack by the rust, and dews that promoted rust intensification often occurred on such sites. Another influential factor, aside from site conditions, was the character of the leaves. Young, thrifty leaves on current season's growth were much more retentive than older leaves, or leaves on wood of previous growth. The last leaves to drop from a plant were always from branch growth of the current season. The infected leaves of *R. roezli* that were the last to drop in the autumn were the tip leaves on current growth of shade-form plants growing on moist sites.

Rust Development

Although the initial infection on ribes in 1938 was somewhat lighter than in 1941 and 1943, because of the greater distance from the aecial source, there were several well-distributed rains in June, 1938, that caused considerable intensification of the rust. By July and August the amount of rust was about equal to the amount present at this same time in 1943. In 1941, however, rains permitting uredial intensification were not only abundant during the spring months but also continued to occur frequently throughout the summer. However, the greater premature leaf-fall of Ribes roezli in 1941, caused by the heavier rust infection, prevented the rust on that species from building up to a much greater amount than in 1938 and 1943. The premature leaf-fall on R. nevadense in 1941 was less excessive, and the rust on that species built up much more than in the other two years. Figure 1 shows the amount of rust, per foot of live stem, present on the ribes plants throughout the 1941 season. Figure 2 shows the development of rust in These figures also show the amount of infected leaf surface bearing telia, the portion of the telia that was viable, and the time that weather probably favorable for pine infection occurred. A considerable number of telia germinated, as a result of the summer rains in 1941, before the leaves were dropped, whereas in the absence of summer rains in 1943 telia usually remained viable until the infected leaves were dropped. Although most telia on leaves of R. nevadense remained viable until rain occurred, some died before; the telia that did not remain viable until the rain of October 11 in 1943 had not germinated but had died, usually as a result of the death of large infected portions of the leaf. Although infected leaf portions died before and after uredia were developed on both ribes species, infected leaf portions bearing telia on R. roezli never died before the leaves were dropped. On some plants of both species practically all infected leaf tissue died before producing telia. Very short, dark, abortive columns of telia, with little or no evidence of viability, were found in 1938 on the small, thick, coarse leaves of a few open-form plants of R. roezli growing on dry sites. Such abortive telial columns were rarely seen in 1941 and 1943. The percentage of the infected leaf surface that produced telia was higher in R. roezli than in R. nevadense and would have been higher still had consider-

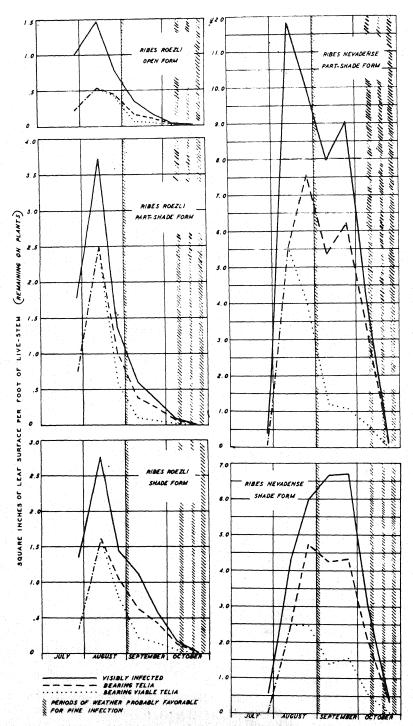


Fig. 1. Average blister rust development on plants of the various light forms of Ribes roesli and R. nevadense at the Montgomery Creek Area in 1941. (All telia were viable in some cases, as indicated by the complete overlapping of the dot and delivery

able uredial infection not been lost by premature leaf-fall. In addition to producing a higher percentage of telia, the infected leaves of R. roezli had nearly twice the number of telial columns per unit of telial-bearing leaf area that the infected leaves of R. nevadense had. Telial counts, through

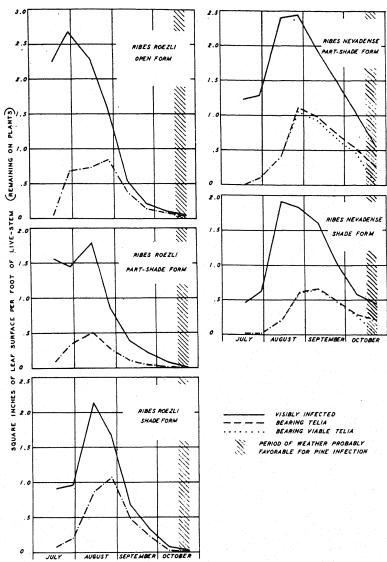


Fig. 2. Average blister rust development on plants of the various light forms of Ribes roezli and R. nevadense at the Hatchet Creek Area in 1943. (All telia were viable in many cases, as indicated by the complete overlapping of the dot and dash lines.)

a microscope, made on 36 typical specimens for each species, showed that an average square inch of telia-bearing leaf surface of *R. nevadense* contained approximately 4,600 telial columns, while that of *R. roezli* contained 8,700 telial columns.

General observations on other naturally infected Ribes roezli and R. nevadense over a number of years and at various localities and data and observations on plants used in other blister rust studies, under controlled infection conditions, have been in accord with the findings herein reported. There is a great variation in susceptibility between plants of R. roezli, greater variation than for any other ribes species tested in the West. Many plants were not infected while adjoining plants were severely infected. Some plants exposed to an abundance of acciospores or urediospores under controlled infection conditions have never become infected in any visible degree, whereas other plants have become severely infected under less favorable conditions. There is an indication, from the various tests made, that in the northern range of R. roezli a greater percentage of plants have this high resistance to infection than in its southern range.

DISCUSSION

For ribes to cause pine infection it is necessary that they have viable telia at the time of a favorable moisture period of sufficient duration to bring about germination of teliospores, the development of the promycelia, the formation and dispersal of the sporidia, the germination of the latter, and the penetration of the germ-tubes into the needles of the host. time required for these processes varies with the air temperature and the age of the teliospores at the time of the favorable moisture period. favorable temperatures 24 to 36 hours are required for appreciable pine infection, during which high humidities must prevail in order to maintain the necessary free moisture (5, p. 33). In most parts of the sugar pine region this would occur only during rainfall, but in small localized areas. following a short but heavy rain, free moisture may be retained sufficiently long on ribes plants and pine foliage that are well shaded by a high canopy and further sheltered from wind and sun by dense surrounding brush and other vegetation. Such areas are on sheltered north slopes or on wet sites where a heavy night dew often follows a heavy rain. Pine probably will not become infected at temperatures below 32° F., and below 50° F. a progressively longer time is required (3). For this reason periods of snowfall usually are not favorable for pine infection, and late autumn rains often occur when the air temperature is so low that the period required for pine infection is much greater.

Moisture for less than 24 hours often induces germination of teliospores without the subsequent infection of pines. Short rains usually intensify the rust on the ribes plants, through germination of urediospores on the leaves, and incite the development of new telia.

Both Ribes roezli and R. nevadense had sufficient viable telia to serve as a source for considerable pine infection at the time of favorable rains in 1938 and 1941; however, in 1943 the light shower on October 11 may have induced germination of some of the few remaining teliospores on R. roezli, without subsequent pine infection, before the favorable rains starting on October 17. Even if this earlier rain did not dissipate teliospores, a smaller amount

of viable telia remained on leaves of R. roezli on October 17 than occurred on this species at the time of favorable rains in 1938 and 1941. Telia remaining on R. nevadense on October 17, however, were still sufficient for considerable pine infection even if partly dissipated by the October 11 rain. Observations in the three years indicate that when there are no summer rains to maintain a supply of uredia it is possible that little or no pine infection will occur if the first autumn rain dissipates the telia, but does not provide ample time for subsequent pine infection. With relatively frequent summer rains, however, it is probable that sufficient uredia will be present to cause intensification of the rust, with the subsequent production of new telia on the ribes, so that there will still remain a chance for pine infection at the time of later rains. If the first rain occurs after mid-October it appears likely that only plants of R. nevadense and the shade form of R. roezli will have enough leaves to provide the requisite inoculum. The success or failure of the rust to infect pines during any year will depend largely on whether or not a rain of sufficient duration occurs before ribes defoliation, whether the defoliation be caused by heavy rust infection, drought and hot weather, or heavy frosts. Under infection and weather conditions similar to those on the study areas in any of the three test years R. roezli will bear adequate inoculum for pine infection at any time from late-July to late-September, and with the shade-form plants growing on moist sites this period may be extended to mid-October. Under similar conditions R. nevadense may provide inoculum for pine infection from early-August to late-October.

It appears then, from all observations, that with heavy rust infection, either from abundant aecial sources, or as a result of weather conditions favorable for rust intensification, per foot of live-stem, *Ribes nevadense* will be the more important host of the two ribes species concerned. Even with less severe infection *R. nevadense* will be of more consequence when the autumn rains are late. Per foot of live-stem, *R. roezli* will be of greater importance only under conditions of less severe infection when summer or early autumn rains occur and are of sufficient duration for pine infection. Blister rust control workers have found that on the average approximately 2,900 feet of *R. roezli* live-stem and 420 feet of *R. nevadense* live-stem occur per acre within the California sugar pine region before eradication. Therefore on average unworked areas it is most probable that the larger population of *R. roezli* plants, and this species' greater production of telial columns per unit of leaf surface bearing telia, will make it the more dangerous one, unless the first rain suitable for pine infection occurs after mid-October.

SUMMARY

As an aid to planning control of white pine blister rust in the California sugar pine region, observations were made on the development and defoliating effect of this disease on naturally infected plants of *Ribes roezli* and *R. nevadense* in their native habitat in the northern Sierra Nevada in 1938, 1941, and 1943.

Infected plants varying in degree of initial infection and growing on

different sites were examined biweekly and detailed data were taken on host and rust development and reaction from July to October, inclusive, each vear.

In general, the susceptibility of the two ribes species to infection by the rust and the telium-producing capacity of R. nevadense were the same as reported from earlier studies in British Columbia and the Pacific Northwest; but R. roezli produced considerably more telia, because fewer infected leaves were dropped before telia were developed. Although R. roezli remained the more susceptible of the two species and its infected leaf area produced a higher percentage of telium-bearing surface and nearly twice the amount of telial columns per unit of telium-bearing surface, the premature dropping of its infected leaves still prevented it from being as dangerous a pine-infecting species as R. nevadense per unit of population. In unworked areas of the California sugar pine region R. roezli is the more potentially dangerous species because its greater population more than counterbalances its limited capacity to retain telium-bearing leaves.

Severity of infection, site conditions, character of infected leaves, and occurrence of autumnal frosts appeared to be the more significant factors influencing premature fall of infected leaves of Ribes roezli. Only severe infection and heavy autumnal frosts caused appreciable premature fall of infected leaves of R. nevadense.

Infected plants of Ribes roezli growing under shade and producing abundant new woody growth, especially plants on moist sites, are potentially the most dangerous of this species with respect to spread of blister rust. The tip leaves of the current season's growth on such plants were the last to drop in the autumn, and consequently urediospores and viable telia remained latest on this type of leaf.

Many infected leaves of Ribes nevadense were present on the plants at the time of the first favorable autumnal rains.

The time of year that rain occurs in sufficient duration to provide for pine infection will determine the success or failure of the rust, from either ribes species, to infect pines in a given year.

DIVISION OF FOREST PATHOLOGY.

UNITED STATES DEPARTMENT OF AGRICULTURE.

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AECIAL HOSTS OF PUCCINIA GRAMINIS IN CHINA

LEE LING!

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The susceptibility and resistance of the Berberidaceae to Puccinia uraminis Pers. have been extensively studied in the Minnesota Agricultural Experiment Station in the United States. In the summarized account by Levine and Cotter,² 28 species of Berberis indigenous to China were listed. among which 27 are susceptible and one apparently immune. In a more recent list of Stakman,³ 8 additional Chinese species were included. In 1905, Baccarini⁴ on the basis of specimens collected from Shensi, China. considered Epimedium, in addition to 2 species of Berberis, as the host of P. graminis. He apparently mistook the aecial stage of Puccinia epimedii (Henn. et Shir.) Miyabe et Ito for P. graminis. As China, especially in the Himalaya ranges, is known to be the native country of a large number of species of Berberis and Mahonia, the knowledge so far gathered regarding the alternate hosts of stem rust appears to be far from complete. Consequently, many specimens representing about 50 species of Berberis and 15 of Mahonia have been examined for rust infection in the following herbaria of China: National Central University: University of Nanking: National Szechuan University: Institute of Agricultural Research, National Tsing Hua University; Biological Laboratory of the Science Society of China; and Szechuan Provincial Agricultural Improvement Institute. Field observations also have been made in the western part of Szechuan province.

As a result of herbarium examination and field observations, 12 species of Berberis and 1 of Mahonia have been found harboring the aecial stage of Puccinia graminis (Table 1). Three of them, B. silva-taroucana Schn., B. virgetorum Schn., and M. fortunei (Lindl.) Fedde, are hitherto unrecorded as carriers and breeders of stem rust. It is even more interesting to note that B. gagnepainii Schn., B. julianae Schn., and B. sargentiana Schn. are classed in the list of Stakman as immune or highly resistant in the spring wheat region in the United States, but conspicuous infections have been noticed on the specimens in China. Another species, B. dielsiana Fedde, according to the results of artificial inoculations made by Levine and Cotter in Minnesota, is attacked only lightly by P. graminis secalis Erikss. et Henn. and not at all by P. graminis tritici Erikss. et Henn. This species, however, is the one most common in the mountain region (sungshan) of northern

Lehmanns Verlag, München/Berlin. 1937.

4 Baccarini, P. Funghi dello Schensi septentrionale raccolti dal Padre Giuseppe Giraldi. App. al. Nuovo Giorn. Bot. Ital. 22 (nuova serie): 689-698. 1905.

¹ Phytopathology extends the courtesy of its journal pages to scientists in other countries who are persevering in research under difficult wartime conditions and are temporarily deprived of the opportunity for membership in the American Phytopathological Society.

2 Levine, M. N., and R. U. Cotter. Susceptibility and resistance of Berberis and related genera to Puccinia graminis. U. S. Dept. Agr. Tech. Bull. 300. 1932.

3 Lehmann, E., H. Kummer, and H. Dannenmann. Der Schwarzrost. 584 pp. J. F. Lehmann, Volley Affirsh (Parity 1997).

TABLE 1.—Aecial hosts of Puccinia graminis in China based upon field and herbarium observations

Species	Localities where rusted specimens were collected	Known distribution in China
Berberis acuminata Fr.	Kunming, Yunnan	West Szechuan, Yunnan
B. amurensis Rupr.	Wulingshan, Hopei	Hopei, Shansi, Mongolia Manchuria
B. dielsiana Fedde	Sunghsien, Honan	Honan, Shensi, Szechuan
B. gagnepainii Schn.	Mt. Omei, Szechuan	West Szechuan, Sikang
B. henryana Schn.		
B. julianae Schn.	Wantsaoshan, Hupeh	Hupeh, Anhwei Hupeh, Yunnan, Shensi
B. l̃evis Fr.	Mt. Omei, Pánlanshan, and Kwanhsien, Szechuan	West Szechuan, Yunnan, Sikang
B. pruinosa Fr.	Chengkiang, Tungchwan, and Süanwei, Yunnan	Yunnan
B. sargentiana Schn.	Suon Nai Ook, Hupeh	West Hupeh
B. silva-taroucana Schn.	Mt. Omei, Szechuan	West Szechuan, Sikang
B. virgetorum Schn.	Kuling, Kiangsi	Kiangsi, Kwangtung
B. wilsonae Hemsl.	Lifan, Szechuan	West Szechuan, Sikang, Yunnan
Mahonia fortunei (Lindl.) Fedde	Mt. Omei, Szechuan	Szechuan, Sikang, Hupel

Honan province and is usually severely rusted by P. graminis. This probably indicates that certain physiologic races are virulent and that those species which have been considered immune or resistant in America probably could not be introduced and distributed there with entire safety. B. sargentiana, which has been recommended⁵ as one of the most desirable for

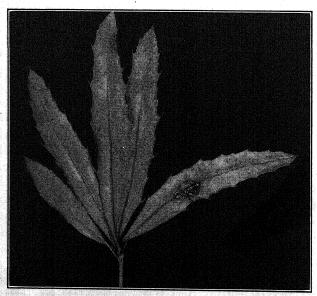


Fig. 1. Aecial infection of Puccinia graminis tritici on Berberis levis.

⁵ Sargent, C. S. (editor). Plantae Wilsonianae. Publication of Arnold Arboretum
No. 4. Vol. 1. 1913. Vol. 3. 1917.

introduction as a garden plant to the United States because it was perfectly hardy in the Arnold Arboretum, probably should be regarded with caution.

The Province of Szechuan is bounded by mountain ranges of moderate height (1,500 to 3,000 meters) mostly; but along the western and north-western edges some extremely high peaks and ranges, mostly unexplored and unsurveyed, appear to be from 5,000 to 7,000 meters high. In those western ranges, the richness of *Berberis* and *Mahonia* is well known. E. H. Wilson alone collected 31 species and varieties of those two genera there. The hollow land in the center of the province has been named by geographers the Red Basin of Szechuan, where wheat is generally harvested in May and stem rust ordinarily comes too late to cause substantial damage. In the mountain regions, however, heavy losses are frequent in both winter and spring wheats.

West of Kwanhsien along the edge of Chengtu plain, in the mountains such as Chinchenshan and Panlanshan, Berberis levis Fr. (= B. soulieana Schn.) is one of the most common shrubs. It has been recorded at higher altitudes, up to 2,300 meters, but commonly it is found below 1,000 meters, mostly along creeks at the foot of the mountains. Early in April every year, the bright red infections of Puccinia graminis on the leaves (Fig. 1) are numerous and startling. The situation is equally as serious as, if not more so than, that of B. vulgaris L. in the north central United States. Omei, southwest of Chengtu plain, Mahonia fortunei is even more common than B. levis. Both species are usually severely infected by stem rust there. The M. fortunei appears to be restricted to the foot of the mountain, between 500 to 800 meters, and extends to the plain. B. gagnepainii, occurring from 1,500 meters to the summit of the mountain (3,100 meters), is less often Seven collections of the rust on B. levis and two collections on M. fortunei were incoulated to grain crops in 1940. The resulting good infection on wheat and barley but feeble infection on rye proved that all the collections belonged to P. graminis tritici. As a matter of fact, wheat and barley are the only small grains in the Chengtu plain infected naturally by stem rust, while oats and rye, the latter grown in the far north of Szechuan province and observed in experimental plots only, have never been seen infected. It is even more unlikely that all the common wild grasses in the Chengtu plain, such as Poa, Agropyron, Agrostis, Sporobolus, Alopecurus, Elymus, and Bromus, are also free from stem rust.

Northwestward, in the mountain ranges of Szechuan, covering Lifan, Maohsien, and Wenchuan, Dr. C. T. Wei observed in 1941 four species of Berberis common at levels around 2,000 meters, i.e., B. wilsonae Hemsl., B. silva-taroucana, B. verruculosa Hemsl. et Wilson, and an undetermined species with round to broadly oval leaves. B. wilsonae has been seen very heavily rusted, B. silva-taroucana occasionally and lightly infected, while the other two species were free from rust.

In view of the common occurrence and the destructiveness of stem rust in many provinces in China, the role played by barberry deserves a thorough

and long study in order to solve the rust problem. The present note, based on limited observations, aims only to stimulate further investigations. It is a pleasure to acknowledge my indebtedness to Dr. C. T. Wei, Dr. C. S. Wang, and Dr. T. F. Yu for their cooperation in furnishing information and specimens.

UNIVERSITY OF NANKING, CHENGTU, CHINA.

STUDIES IN THE FUSARIUM DAMPING-OFF OF CONIFERS. I. THE COMPARATIVE VIRULENCE OF CERTAIN FUSARIA^{1, 2}

HOWARD TINT3

(Accepted for publication January 15, 1945)

INTRODUCTION

In the many reports of the relationship between Fusaria and damping-off in conifers, either observed or determined by inoculation experiments, the repeated occurrence of certain species suspected of being causal agents has led to the general acceptance of the pathogenic nature of a few of these. According to a compilation by Wollenweber and Reinking (32), of importance in Europe are Fusarium oxysporum v. aurantiacum, synonymous with Fusoma parasiticum Tubeuf (4, 29, 30, 31) and Fusoma pini Hartig (2, 9, 10), F. oxysporum v. aurantiacum f. 1 (F. macroxysporum Lindfors) (16), F. bulbigenum v. blasticola (F. blasticola Rostrup) (24), F. culmorum, F. avenaceum (F. venenorum) (6), F. sambucinum, F. scirpi v. acuminatum. F. fuligenosum and F. echinosporum (25), and F. graminearum (Giberella saubinetii) (19); and in the United States primarily F. sporotrichioides, F. arthrosporioides, F. sambucinum f. 6 (F. discolor sulphureum Schl.), and to a lesser extent F. culmorum, F. solani, F. orthoceros, F. moniliforme, F. oxysporum, F. vasinfectum and F. graminearum (12, 13, 20, 21, 26). These constitute only a small selection of the many species mentioned in the literature, and there is need for further tests with some of them as well as with other Fusaria, in order to determine which species are really parasitic and how they compare with other damping-off fungi used as standards. object of the present investigation has been to carry out such tests by various critically studied experimental methods. Where more than one host was inoculated, relative susceptibility to infection was tested.

The effect upon the experimental design of limitations in time and space made it infeasible to consider damping-off losses according to the classical symptomatic categories (12, 13, 28): germination loss, normal or ordinary damping-off, root-rot or late damping-off, and top damping-off. Experiments were maintained long enough to test ability to induce normal damping-off, but probably not long enough to test potentialities of the Fusaria as root-rotting organisms. Damping-off losses were in two classes: (I) emergence loss, the reduction in the number of seedlings appearing above the surface of the substrate as compared with the total number of viable seeds, the latter determined by the emergence in uninoculated controls. This

³ The greater part of this investigation was carried on during the tenure of a George Leib Harrison Fellowship at the University of Pennsylvania.

¹ A portion of a dissertation presented to the faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

² The writer wishes to express appreciation to: Dr. H. H. York under whose direction the investigation was carried out; Dr. W. G. Hutchinson for criticism of the manuscript; Dr. P. V. Lufkin for assistance on statistical analysis; and Dr. C. D. Sherbakoff for identifications of some Fusaria used in the investigations.

TABLE 1.—Identity and sources of fungi tested for ability to cause damping-off of coniferous seedlings

Species	Line Host source	Year of isolation	Locality	Isolatorb
Fusarium aquaeductuum (Radlk.	1Cc Red pine (discolored wood of stem, 16-vrold tree)	f 1932	Hemlock Lake, N. Y.	Y
F. arthrosportoides Sherb.	Χ	1940	Washington Crossing, N. J.	EH E
T. avenaceum (FI.) Bacc.	20 00 00 00 00 00 00 00 00 00 00 00 00 0	. , ,,	27 27 27 27 27 27 27 27 27 27 27 27 27 2	- E-
	97 97 97 97 97 97 97 97 97 97 97 97 97 9	3)	23 23 23 23	E
				H E
F. equiseti (Cda.) Sacc.	White pine (damping-off)))))	Philadelphia, Pa. Weshington Crossing N. I	×ΜΕ
F. javanicum Koord. v. radici-			Hashington Crossing, 11. 0.	→
cola Wr.	13. 11 11 11 11 11 11 11 11 11 11 11 11 11			
F. orthoceros App. & Wr.	M	f 1937	Hemlock Lake, N. Y.	Ā
	R23Ae Red jine (root rot, 1-2-yrold	d 1939	Saratoga Springs, N. Y.	闰
F. orthogeros App. & Wr. v.	securings) 28c White pine (damping-off) R60 Secteh pine (damping-off)	1940	Philadelphia, Pa. Washington Grossing, N. J.	ML
longius (Sherb.) Wr.))))))))))))))))))))))))))	T
F. oxysporum Schl. F. poae (Peck) Wr.	Ç BA	h 1936	Hemlock Lake, N. Y.	Y
	(15), in 1935 node) 68c Scotch pine (damping-off)	1940	Washington Crossing, N. J.	L
		The second secon	THE PERSON NAMED ASSOCIATION OF THE PERSON NAMED IN COLUMN TWO IS NOT	

TABLE 1.—(Continued)

Species	Line	Host source	Year of isolation	Locality	Isolatorb
F. reticulatum Mont.	26A 58e	White pine (damping-off) Scotch pine (damping-off)	1940	Philadelphia, Pa. Washington Crossing, N. J.	MHH
F. sambucinum Fuckel	$^{62}_{ m R8A^{\circ}}$	Norway spruce (wood 1-in, root	1937	Hemlock Lake, N. Y.	X
F. scirpi Lamb. & Fautr. v.	R22c	Red pine (stem wood of 1925	1931	33 33 33 33	X
acuminatum (Ell. & Ev.) Wr. F. solani (Mart.) App. & Wr.	R9B°	node) White pine (inner bark of root	1932	Norwich, N. Y.	Y
F solani (Mart.) v. Martii	R2Ae	Crown) White pine (wood of $\frac{1}{5}$ -in. root	1936	2)))	X
(App. & Wr.) f. 1 Wr. F. solani (Mart.) App. & Wr. v.	67	of 22-yrold tree) Scotch pine (damping-off)	1940	Washington Crossing, N. J.	H
minus Wr. F. sporotrichioides Sherb.	R33	American Type Culture Coll.		- Annual Control of the Control of t	
F. vasinfectum Atk. Penicillium sp. Define officiam Trow	K34 CI 1145	Greenhouse soil Douglas fir (damping-off)	1940 1925	Philadelphia, Pa. Monument, Colorado	E Hol
Rhisoctonia solani Kühn Sclerotium bataticola Taub.	631 573	Tulip poplar (collar rot) Red pine (collar lesion)		Frederick Jc., Maryland	ى 1

^a Nomenclature after Wollenweber and Reinking (32).

^b Isolations by: Y, Dr. H. H. York, Department of Botany, University of Penna.; E, E. J. Eliason, State Department of Conservation, Saratoga Springs, N. Y.; K, P. D. Keener, Dept. of Botany, University of Penna.; T, the writer; J, L. W. R. Jackson, B.P.I.S. & A.E., U. S. Dept. of Agriculture.

eldentified by C. D. Sherbakoff, Univ. of Tenn. Agricultural Experiment Station.

class thus included the decay of seed as well as the early rotting of radicles. (II) Post-emergence damping-off, the reduction of the total number of emerged seedlings through normal damping-off, top damping-off and root rot, all cases of infection being tabulated only if fatal before the termination of the experiment. The two categories could then be summarized in a survival count, based upon the number of seed planted, which, when contrasted with an uninoculated control, would provide a measure of the whole effect of the damping-off organism for the duration of the experiment.

MATERIALS AND METHODS

Isolations were made by dipping infected portions of diseased plants in four per cent formalin for two minutes and then placing them without rinsing upon two per cent agar without nutrients. A modification of this method was used with the fungi which were secured under conditions of pure saprophytism or whose origins were not known. Surface sterilized seeds of *Pinus resinosa* Ait. were sown on agar cultures of such fungi and then isolations were made from seedlings which damped-off after germination. With this method each fungus invariably produced a certain amount of injury, as demonstrated either by the decay of radicles or normal damping-off. Passing fungi of unknown pathogenicity through a potential host permitted the selection of more virulent strains, which could then be compared with other fungi isolated from authentic cases of the disease.

Monosporous transfers were made with a micromanipulator. Every monosporous culture coming from diseased seedlings, either directly by isolation or indirectly from passage through a host, is called a line throughout this paper. Stock cultures were maintained on potato-dextrose agar (Difco, pH 5.6) in cold storage at 8° to 10° C. or at room temperature from 18° to 25° C. A list of the names and sources of the fungi tested is given in table 1.

The selection of hosts for pathogenicity tests was based upon a compilation of susceptible conifers listed by Hartley (12). The list was restricted to abietinean conifers owing to their economic significance in reforestation and to the frequency of reports of disease among them. All seeds were kept in dry, unsealed containers in a cold-storage room at 8° to 10° C. A list of the species in the various inoculation tests is given in table 2.

Inoculations were made in liquid, quartz-sand, and autoclaved-soil cultures. All seeds, before planting, were surface-sterilized by immersion for 3 minutes in an aqueous solution of 0.1 per cent bichloride of mercury, followed by at least 5 rinses in sterilized distilled water.

Inoculum was prepared by growing the fungus on a substrate of 25 cc. of polished rice grains in 35 cc. of distilled water autoclaved for 20 minutes at 15 pounds' steam pressure. This sterile rice-mush was thoroughly permeated by the average Fusarium in 3 weeks at 28° C. Unless otherwise indicated, the inoculum was added to the substrate upon which the hosts were to be grown, at the approximate rate of one cubic centimeter per 5 square inches of surface. After the inoculum was mixed with the upper

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inch of substrate, this entire layer was removed, thoroughly mixed in a mortar and replaced upon the surface of the pot or flat. This provided for uniform distribution of the inoculum in the upper inch of substrate, as well as for uniformity of age and quantity of inoculum of the different funci. In the case of the liquid cultures, however, it was necessary only to add to the substrate a single hyphae-permeated rice grain of inoculum.

For those experiments in which liquid or quartz-sand media were used, a nutrient solution was selected which would be stable under emendation for pH and nutrition study and, at the same time, adequate for the growth of both hosts and pathogens. Glycerinated phosphates met these require-The advantages of substituting a salt of glycerophosphoric acid for that of phosphoric in standard nutrient solutions have been described (1). The solution ultimately adopted was a modification of a glycerophos-

TABLE 2.—Seed source of conifers used in damping-off trials

$Species^a$	Year of collection	Locality of collection ^b
Pinus resinosa Ait.	1932	Adirondack region, N. Y. (a)
Pinus sylvestris L.	1938	Brushton CCC Camp, N. Y. (a)
Abies concolor Lindl. & Gord.	1940	San Isabel Forest area, Colorado (b)
Pinus nigra Arnold v. aus-		
triaca Aschers & Graebn.	1940	Illinois (b)
Picea pungens Engelm.	1940	San Isabel Forest area, Colorado (b)
Pinus ponderosa Dougl.	1940	Lewis and Clark N. F. Mont. (c) (4000 ft., kiln extracted)
Pinus banksiana Lamb.	1937-38	Superior, N. F., Minn. (c)
Pseudotsuga taxifolia Brit.	1939	Mt. Baker Forest, Darrington, Wash. (c)

phate type successfully used by Jackson (14) for the growth of Douglas Fir and Pinus ponderosa, as well as various isolates of Pythium and Rhizoctonia. The concentrations of the constituent salts were as follows: calcium nitrate, 0.007 M: magnesium sulphate, 0.04 M; sodium glycerophosphate, 0.04 M; potassium chloride, 0.01 M; iron citrate, to yield approximately 2 ppm. (1 ml. 1 per cent solution); boron (boric acid), 0.05 ppm.; copper (sulphate), 0.02 ppm.; manganese (chloride), 0.5 ppm.; and zinc (chloride), 0.05 ppm. The salts were made up in stock solutions, sterilized by autoclaving, and added to distilled water in the proper quantities to make the required concentrations as needed. The reactions of the solutions were adjusted to desired endpoints with 0.1 N HCl and 0.1 N NaOH. All pH determinations were made potentiometrically with quinhydrone and saturated calomel halfcells.

INOCULATION EXPERIMENTS AND RESULTS

Liquid Cultures

In preliminary tests, Pinus resinosa grown upon sterile liquid medium in test tubes was inoculated with a number of fungus lines (Fig. 1), following

^a Taxonomy of conifers after Rehder (22). ^b Seed received from: (a) E. J. Eliason, State Tree Nursery, New York State Conservation Department, Saratoga Springs, N. Y.; (b) Herbst Bros., New York City; (c) H. L. Shirley, Allegheny Forest Experiment Station, Phila., Pa.

with some modification the technique described by van Luijk (17) for grasses and applied to conifer study by Ten Houten (28). A hollow cylinder of filter paper, with the upper end covered by a lid bearing six 2-mm. holes

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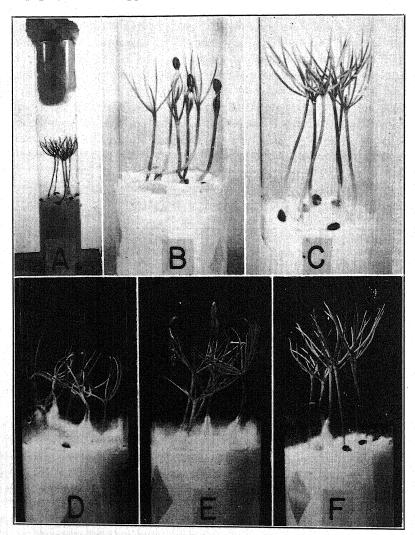


Fig. 1. Inoculation of seedlings of *Pinus resinosa* with lines of *Fusarium* in sterile liquid medium.

Control series: A. View of entire culture tube showing arrangement of seedlings. B. Enlarged view of sterile seedlings, 8 days after transplanting. C. Sterile seedlings 30 days after transplanting.

Inoculated series at termination of experiment (21 days). D. Fusarium orthoceros v. longius (R60), all seedlings attacked. E. F. moniliforme (R35), all attacked. F. F. vasinfectum (R34), 3 seedlings to right not attacked and without typical constrictions.

regularly distributed along the periphery and a larger aperture (5 mm.) in the center, was inserted into the bottom of a test tube $(3.5 \times 20$ cm.). Exactly 50 cc. of nutrient solution, adjusted to yield pH 6 after sterilization,

was added so that the surface of the liquid reached the level of the lid. The tube was then plugged and autoclaved. Seedlings from surface-sterilized seed, germinated in sterile agar and closely checked for sterility after germination and with radicles from one to three cm. long, were transplanted to the tubes with aseptic precautions. The radicle of a seedling was inserted through one of the peripheral holes in the lid of the cylinder. The cultures were placed in a greenhouse (thermostatic control, 68°–75° F.) for three days. Seedlings that failed to grow were removed and replaced. When all seedlings were growing satisfactorily, the tube was inoculated by inserting a grain of inoculum into the liquid through the central hole in the lid. A small quantity of glass wool within the cylinder served to lodge the inoculum

TABLE 3.—Damping-off of Pinus resinosa seedlings inoculated with various fungi in liquid cultures

		Number of	Number of seedlings invaded		
Line no.	Fungus	seedlings - inoculateda	After 10 days	After 21 days	
R11C	Fusarium poae	12	12	12	
R34	F. vasinfectum	18	10	14	
R33	$F.\ sporotrichioides$	36	27	35	
R60	F. orthoceros v. longius	18	12	18	
16	$F.\ orthoceros$	18	17	18	
64	F. avenaceum	12	12	12	
R32	F. javanicum v. radicicola	18	6	9	
58	F. reticulatum	12	12	12	
1C	F. aquaeductuum v. medium	12	3	7	
R8A	F. sambucinum	18	5	7	
R35	F. moniliforme	18	11	18	
R2A	F. solani v. martii f. 1	12	3	- 8	
631	Rhizoctonia solani	$\overline{12}$	12	12	
Control		24b	0	0	

a Six seedlings per tube.

just below the surface of the liquid, as well as to provide support for the seedlings. Holes cut in the sides of the cylinder permitted the observation of the development of the host and the fungus. The inoculated tubes were kept for 3 weeks in the greenhouse.

The criterion of whether a fungus was able to attack the host was the visible evidence of a constriction in the root or hypocotyl, where the cortical tissue was destroyed. A collapse of the seedling, the typical normal damping-off symptom, could not always be used because the seedling received considerable support from adjacent plants and from the wall of the tube; nor could fungal growth along aerial portions of the plant be used as a sign of infection, since this commonly occurred because of high humidity. However, from seedlings with typical constrictions (Fig. 1, D, E and F), surface-sterilized and sown on potato-dextrose agar, the inoculated fungus could be isolated. Plates from unconstricted seedlings, with the same treatment, invariably remained sterile.

b An additional control tube showed a mold several days after the seedlings were transplanted. Two seedlings were rotted at the expiration of the test (21 days).

All 12 of the Fusaria in the liquid-culture inoculation tests were pathogenic (Table 3). Fusarium avenaceum, F. poae, F. moniliforme, F. reticulatum and the two varieties of F. orthoceros caused complete losses, comparing thus in virulence with the Rhizoctonia line. F. sporotrichioides was also highly parasitic. The rest of the lines were moderately parasitic. F. sambucinum was the least virulent of the lines tested in this experiment.

Quartz-Sand Cultures

The quartz-sand cultures (Fig. 2) were maintained in four-inch pots with sand that had been washed for several hours in cold, running water, soaked in hot water, and then rinsed in distilled water. Each pot was painted on all surfaces with DuPont acid- and alkali-resistant black paint to

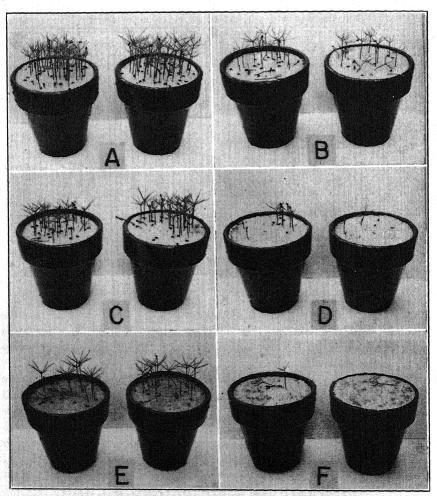
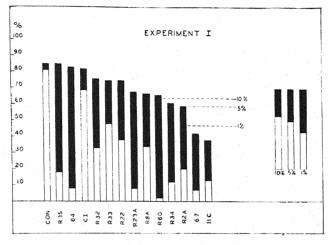


Fig. 2. Inoculations in quartz-sand cultures of Pinus resinosa with lines of Fusarium, photographed 26 days after planting and 14 days after emergence: A. Controls. B. F. orthoceros v. longius (Line 61). C. F. poae (Line 68). D. F. reticulatum (Line 58). E. F. oxysporum (Line 30). F. F. avenaceum (Line 66).

reduce water loss through evaporation. The pots of sand were covered with Petri-dish lids and autoclaved for one hour at 15 pounds. After inoculation, the pots were flooded with the standard nutrient solution adjusted to pH 6. and were incubated at room temperature for several days to permit thorough permeation of the sand by the fungi. Each line was inoculated in duplicate and controls were prepared with sterile rice-mush. Fifty surface-sterilized



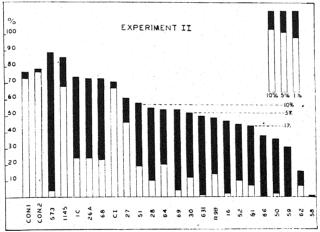


Fig. 3. Diagram showing the results of inoculations of quartz-sand cultures of *Pinus resinosa* with various fungi: comparative emergence (length of bar), post-emergence damping-off (black) and final stand (white). Levels of significance for emergence (dashed lines) and post-emergence losses (black portions of bars to right) are given.

seeds of *Pinus resinosa* were then sown on the surface of each pot and covered with one-quarter inch of sterile sand. The pots were kept in the greenhouse and watered daily by flooding with nutrient solution freshly prepared by adding sterile stocks to distilled water, without autoclaving. The glass covers were removed after the seedlings began to emerge from the sand.

All the fungus lines were tested in two experiments, and although the

first was started two weeks before the second, each was maintained for three months under identical greenhouse conditions. The seedlings which damped-off after emergence were removed as soon as recognized and the numbers recorded. The results are in figure 3. The error variance, or the differences in results derived from each of a pair of duplicate pots in a treatment, was statistically employed for determining the significance of the variance between lines and between each line and the control used as a standard (18, 23).

A certain amount of post-emergence loss was evident in all the control series, although relatively small. This was unavoidable, since no precautions were taken to prevent contaminations by air-borne organisms, and in the duration of the experiment some managed to enter the controls. Fusaria were isolated less than once out of eight times from control seedlings which damped off.

Sterilized-Soil Cultures

Inoculation tests in soil were performed on all the coniferous hosts with a selection of Fusarium lines and the fungi used as standards (Fig. 4). The soil contained three parts of loam, two of sand, and two of leaf-mold. After steam sterilization the final pH was 5.8. The soil was placed in wooden flats, with inside dimensions of $9\times12\times5$ inches and each was autoclaved for one hour at 15 pounds pressure on two successive days. Following sterilization, heavy paper covers reduced air contamination until seedlings began to emerge, after which the covers were removed. Flats were inoculated, and controls were prepared, as were pots for the sand-culture experiments. The general thinning of stands (Fig. 4), as compared with the control, indicated a uniform distribution of inoculum.

Three rows of 50 or 60 seeds of each host, depending upon the relative size of the seed, were sown in each flat, making the density of sowing in soil comparable with that in the sand experiments. Each flat was watered daily by top-irrigation with distilled water. The tests were maintained for four months in the greenhouse (daily mean temperature, 68-80° F.), after which the surviving seedlings were removed, the flats resterilized and the entire set of inoculations repeated. Thus the duplicate set in this case was not run concurrently. However, the greenhouse conditions under which both sets were maintained, the first in the spring and early summer and the second in the fall of that year, were made as closely alike as possible and there was no variation in the standard inoculation and planting procedures. The results of the inoculations are given quantitatively in figure 5, each percentage value being the average of the two trials. Some damping-off again occurred in the controls, but as in previous experiments, only a relatively small percentage of the isolations from these seedlings yielded Fusarium species. losses caused by this accidental contamination were extremely small and insignificant in comparison with those in the inoculated flats.

No attempt was made to evaluate the virulence of the various fungi on the basis of a consideration of each host-fungus combination. As is evident from the results, most of the lines showed variability in virulence within themselves on the different conifers. Some lines were fairly consistent: for instance, *Fusarium poae* (Line 11C) and *F. reticulatum* (Line 58) repeatedly reduced emergence of all the hosts to 10 per cent or less of seed sown;

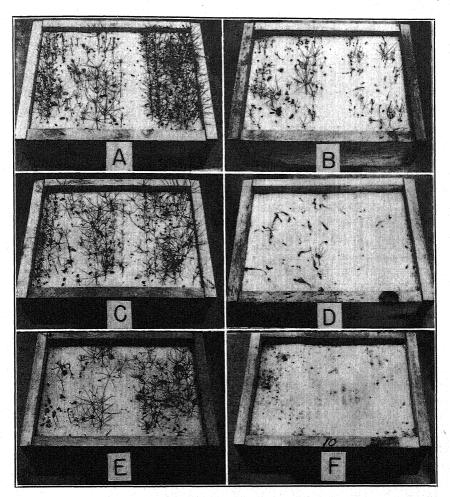
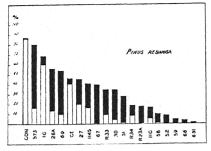
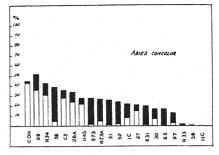


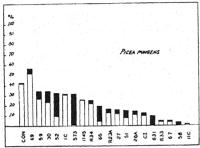
FIG. 4. Inoculations in autoclaved-soil cultures of coniferous hosts with various fungus lines. Hosts arranged in groups of three rows, left to right: Pinus resinosa, Abies concolor, Picea pungens, Pinus ponderosa, Pinus banksiana, blank (originally planted with seeds of Pinus strobus L. but seeds germinated too late and in too few numbers to be comparable with other hosts), Pinus sylvestris, Pseudotsuga taxifolia and Pinus nigra austriaca. A. Control, 28 days after planting. B. F. equiseti (Line 27), 22 days. C. Sclerotium bataticola (Line 573), 28 days. D. F. avenaceum (Line 66), 20 days. E. F. orthoceros (Line 23A), 26 days. F. F. reticulatum (Line 58), 23 days.

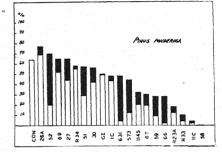
while F. avenaceum caused severe or complete post-emergence losses of all the hosts. Another means of analyzing the data was to consider the pathogenicity of each fungus on all the hosts simultaneously, expressed in terms of the mean emergence or mean post-emergence damping-off that each caused

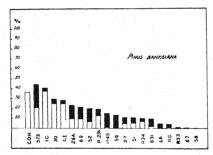
on all the conifers. Conversely, from the same data, it was possible to measure the relative susceptibility of each host to all the fungi and express it as

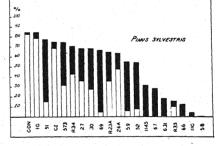


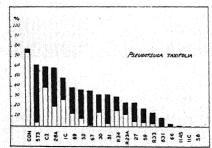












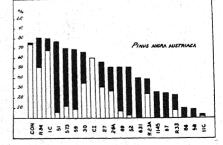


FIG. 5. Diagram showing results of inoculations with various fungi in sterilized-soil cultures of conifers: emergence (Length of bar); post-emergence damping-off (black); final stand in percentage of seed (white).

a mean value. The significance of the differences between such means was determined by analyses of variance, subdividing the variance into three

 ${\bf TABLE~4.--} Total~percentage~emergence~of~all~hosts~in~autoclaved-soil~cultures~inoculated~with~various~fungus~lines$

	Average percent hosts for ea	Order of ability	
Fungus line	Actual means Means based upon controls evaluated at 100 per cent		to reduce emergence
58	2.9	4.2	1
11C	4.1	6.2	ī
R33	13.2	17.6	$\frac{1}{2}$
66	12.4	22.3	$ar{3}$
67	22.2	31.2	4
631	19.6	32.4	$ar{4}$
1145	24.9	41.7	$\bar{5}$
R23A	28.3	45.2	6
27	35.7	53.1	7
59	32.6	53.7	7
52	40.3	59.9	8
R34	42.0	65.9	9
30	42.2	67.5	9
CI	46.0	69.9	10
26A	47.5	72.1	11
1C	53.1	82.8	$\overline{12}$
69	50.7	83.5	$\overline{12}$
573	54.4	84.8	$\overline{12}$
Con	64.2	100.0	*****
east significant differ-			
ence between means	1.3	2.2	

portions: among fungi, among hosts, and between duplicate series of tests (18, 23).

These analyses revealed extremely high variability among fungi and among hosts. In considering emergence data, a slight significance was apparent in the variance due to duplication; however, upon evaluating the emergence in the controls for each series at 100 per cent and reanalyzing the emergence in the inoculated flats in terms of percentage of control, the significance for duplicate trials disappeared. Thus the general effects of the

TABLE 5.—Total percentage emergence of each host in autoclaved-soil cultures inoculated with various fungus lines

Host —	Average percenta hosts for a	Order of susceptibility to emergence	
nost	All lines	Fusarium lines	loss by Fusaria
Pinus resinosa	36.5	33.3	1
Pseudotsuga taxifolia	35.9	34.4	1-2
Pinus banksiana	44.5	38.3	2
Picea pungens	48.2	48.6	3
Abies concolor	53. 0	50.6	3
Pinus sylvestris	57.4	56.8	4
P. nigra austriaca	61.7	60.8	4-5
P. ponderosa	63.9	62.9	5
Least significant difference between means of all lines		4.6	

TABLE 6.—Total percentage post-emergence damping-off of all hosts in autoclavedsoil cultures inoculated with various fungus lines

Fungus line	Average emerg hosts for	e percentage post- gence loss of all r each fungus line		Order of abil to cause pos emergence lo	st-
631		95.8		1	
66		94.0		1	
		83.8		2	
		82.4		2	
67		78.2		3	
573		77.6		3	
59		76.4		3	
51		64.2		4	
R33				<u> </u>	
1145		58.5		5 - 6	
69		55.9		0-0	
R23A		52.8		0-7	
30		50.5		7	
27		44.8		8	11,
26A		39.9		9	1.4
R34		34.4		10	
1C		21.6		11	
CI		13.5		12	
Con		2.2			
Least significant difference			3.7		
between means			3.1		

fungi in the two trials were fundamentally alike; the general emergence potential differed, a condition probably arising from unavoidable variations in the extremely critical sterilization treatment of the seeds. The assumption of optimum germination in the controls permitted a comparison of host reactions to the fungi apart from the different capacities of the seed for germination.

On the basis of these analyses, the results of the soil-culture experiments could be arranged in orderly sequences. The average percentages of emergence of all the hosts for each fungus treatment are given in table 4 in order of increasing sprouting, and the actual means as well as those based on con-

TABLE 7.—Total percentage post-emergence damping-off of each host in autoclavedsoil cultures inoculated with various fungus lines

Host	Average person emergen for f	Order of sus- ceptibility to		
	All lines	Fusarium lines	post-emergence loss by Fusaria	
Pinus resinosa	65.8	75.9	1	
Pseudotsuga taxifolia	69.9	73.4	1 .	
Pinus nigra austriaca	62.8	65.2	f 2	
Abies concolor	59.4	65.1	2	
Pinus sylvestris	57.0	58.6	3	
P. banksiana	51.1	52.3	4	
P. ponderosa	39.7	39.1	5	
Picea pungens	35.7	31.5	6	
Least significant difference between means of all lines		5.6		

trols equal to 100 per cent emergence are included. In addition, the lines are ranked on the basis of their ability to reduce emergence. In table 5 is a like arrangement of hosts, based upon the average emergence after inoculations with all fungus lines, and with Fusarium lines alone. The hosts were ranked in order of susceptibility to emergence loss from the Fusaria. Similar rankings with respect to post-emergence losses are in tables 6 and 7.

DISCUSSION

The results of these inoculation experiments with various lines of fungi, predominately Fusaria of parasitic and saprophytic origin, on a general coniferous population, indicated that certain species of Fusarium were capable of causing extreme reductions in emergence of the total viable seeds of the hosts (Figs. 3 and 5). In some cases the losses were greater than those caused by the standard lines of Rhizoctonia, Sclerotium, and Pythium. Furthermore, some Fusarium lines were comparable with these standards in their ability to cause post-emergence damping-off. In the two sand-culture experiments, 15 of 29 different Fusarium lines tested, significantly reduced emergence in comparison with the respective control series, and 8 of these were beyond the 1 per cent level of probability. Three additional lines produced reductions of questionable significance. All the lines save F. equiseti caused significant post-emergence losses, the exception occurring within the doubtful probability level. Of the standard lines, a common mold, Penicillium sp., and the Pythium line, in both experiments neither reduced emergence nor caused significant post-emergence loss. Sclerotium bataticola produced no emergence loss but did cause a high percentage of post-emergence damping-off, and Rhizoctonia solani was highly virulent both before and after seedling emergence.

Different isolates within the species Fusarium orthoceros, F. reticulatum, F. avenaceum, and F. poae, varied in ability to cause emergence losses in conifer seedlings.

In the soil experiments all the lines tested significantly reduced emergence of the hosts when compared with uninfected controls. Fusarium caused greater emergence losses than did the lines of Rhizoctonia Six additional Fusaria ranged in virulence from a degree comparable with these standards to one resembling the Penicillium. tium bataticola reduced emergence the least of all the lines tested. caused significant post-emergence losses when compared with the control. Rhizoctonia solani and Fusarium avenaceum caused the greatest losses. From the high rate of mortality of the few seedlings which germinated in inoculations with Fusarium poae (Line R11C) and F. reticulatum (Line 58), these lines appeared capable of inducing post-emergence losses comparable with emergence losses; however, for reasons already stated, these were omitted from the analysis. Three Fusarium lines were more virulent than the Sclerotium, and six were more virulent than the Pythium. F. aquaeductuum v. medium caused the least damage of the Fusaria, and the Penicillium less than any of the lines.

Failures of emergence in coniferous nursery beds have long been associated with damping-off organisms; however, species of Fusarium on the whole have generally been credited with less ability to cause pre-emergence than post-emergence losses (11, 12, 13, 28). Results of the present experiments indicate relatively equal virulence for Fusaria in both aspects of this disease, and agree with other observations (8, 21) that many Fusaria may reduce sprouting by the early decay of radicles. One author has stated that Pythium ultimum and virulent strains of Corticium (Rhizoctonia) solani apparently caused more germination losses of conifers than any Fusarium line tested, with the exception of F. sporotrichioides (21). The present investigations in soil and sand substrates, particularly the latter, indicate to the contrary that a number of Fusarium lines may reduce emergence to a

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Rather high emergence losses and post-emergence losses consistently resulted from inoculations with Fusarium reticulatum (Line 58), F. poae (Line R11C), F. avenaceum and F. sporotrichioides; moderate losses of both types followed inoculations with F. vasinfectum and F. oxysporum; but F. aquaeductuum v. medium caused little loss of either kind. Other lines were less consistent: some caused considerable emergence reduction with relatively little subsequent attack; others reduced emergence comparatively little, but caused high post-emergence losses.

greater extent than the species mentioned.

The virulence of the fungi depended to a very large extent upon the conditions under which they were tested. In liquid culture inoculations in test tubes, where only post-emergence attack was considered, all the lines caused appreciable or complete destruction of seedlings, even the relatively nonvirulent Line 1C. The conditions set up favored the fungus to such extent that even the least virulent organism attacked the host in the unnatural environment. Fine distinctions of relative virulence are not obtained by this method. In quartz-sand cultures, where the host had a solid substrate, approximately half the lines significantly reduced emergence while almost all caused heavy post-emergence losses. Here again conditions favored the fungi to the extent that the severity of most of the losses permitted little comparison of relative virulence of the fungi.

The soil-culture method, wherein most of the lines caused both types of loss, represents the closest approach in the laboratory to a duplication of nursery conditions. However, some artificiality is again evident, particularly in the sterilization of the soil, a procedure which is essential in order to remove potential damping-off organisms or any others that might affect the activity of the test organism, but which, as shown by Lindfors (16), unquestionably increases the severity of Fusarium attack. The generally greater emergence in the sand cultures compared to that in soil could not be explained as inability of the fungus to thrive in the sand, since sufficient rice-mush was carried along and uniformly distributed with the fungus mycelium in the process of inoculation so that ample nutrition was afforded the fungi. In addition, the fungi received accessory nutrient material in

the sand cultures from the standard nutrient solutions supplied. The poorer retention of moisture by the coarse particles of sand, with consequently better aeration, probably stimulated emergence and allowed the fungi less opportunity to act upon radicles. These results paralleled Spaulding's observation (27) that the use of a coarse sand cover apparently increased resistance to the disease.

The simultaneous inoculation of all hosts as in the sterilized-soil tests, permitted a valid comparison of their relative susceptibility to Fusaria. Emergence and post-emergence losses were greatest in Pinus resinosa and Pseudotsuga taxifolia. Hartley's (12) observations have indicated, somewhat differently, that Pinus resinosa is more susceptible to the later forms of damping-off than to germination losses. However, from his compilation of the literature on relative susceptibility of conifers, there is general agreement with the present results for P. resinosa, most of the reports indicating a relatively high degree of susceptibility to damping-off. Pseudotsuga taxifolia frequently has been reported as somewhat less susceptible, although later work (5) describes severe attacks on this species. Rathbun-Gravatt (21) had comparable results on P. resinosa and P. banksiana, the seeds of the former showing reduced germination percentages in the presence of Fusarium, a result compatible with those of the present investigation. P. banksiana proved somewhat less susceptible to post-emergence losses. Abies concolor was of moderate relative susceptibility to both categories of losses, somewhat at variance with early reports of its extremely high susceptibility to damping-off (3, 4). Hartley (12) states that literature reports indicate relative susceptibility of *Pinus ponderosa* and resistance of *Picea pungens*. The present results agree with the latter observation. P. pungens, although moderately attacked before emergence, suffered the least post-emergence loss of the conifers tested. Pinus sylvestris has variously been reported as ranging from less susceptible than average to most susceptible (12). The present tests have indicated its relatively intermediate response to both types of attack.

In all these experiments, the virulence of the fungi probably depended to a considerable extent upon the nature of their nutrition prior to inoculation. Fusaria grown upon rice decoction have been more virulent than the same organisms from nutrient agars (20, 28). This is probably due to a higher production by the fungus of toxic materials on the rice, as suggested by the investigations of Ten Houten (28) and of van Eek (7), who is cited by the former to have secured a stronger attack with the extract of a Fusarium culture on rice than with the living fungus itself. Hence, in all the experimental methods used in these studies, the employment of moderately heavy, toxic inoculum, dense sowing, abundant moisture, and greenhouse conditions unquestionably favored the development of the fungi to an extent far exceeding that which would normally be encountered in the field; and probably the magnitude of the losses seen in the tests would ordinarily not

be met in the nursery. However, the possibility remains that under some circumstances, conditions might become similarly favorable for the establishment of comparable host-pathogen relationships. Thus species of *Fusarium*, normal saprophytes in the nursery, might suddenly become highly virulent.

SUMMARY

Four fungi used as standards and monosporous cultures of twenty-five Fusarium lines, some parasitic and others saprophytic, were tested for ability to cause damping-off of coniferous species. A method of host-passage to determine pathogenicity is described. Eight coniferous hosts were selected for inoculation tests.

Losses are tabulated as: (I) emergence loss, including seed decay and destruction of radicles before emergence of seedlings from the substrate; and (II) post-emergence loss, including normal damping-off, top damping-off, and all root-rot fatal before the termination of the experiment.

The relative values of some standard inoculation procedures for testing relative virulence is discussed.

In liquid-culture inoculation tests in glass tubes, of twelve lines of Fusarium tested on seedlings of Pinus resinosa, six caused complete loss, comparing in virulence with a standard line of Rhizoctonia solani.

All of the lines were tested in quartz-sand cultures of *Pinus resinosa* seedlings. Approximately half of the Fusaria reduced emergence significantly compared with the uninfected control, and all save one caused heavy post-emergence losses, the significance of the exception being questionable. Of the standard lines: *Sclerotium bataticola* did not lower emergence but caused high post-emergence losses; *Rhizoctonia solani* effected severe losses in both categories; and *Pythium ultimum* and *Penicillium* sp. did not significantly reduce emergence or cause post-emergence losses.

Inoculation tests in sterilized-soil culture gave the most statistically reliable results of the methods employed because of the higher degree of replication available for analysis. The relative virulence of fifteen Fusarium lines and four standard cultures for a general coniferous population, represented by the eight hosts, was determined; and conversely, the range of susceptibility of the hosts to the entire group of damping-off fungi was ascertained. A number of the Fusarium lines equalled and occasionally exceeded in virulence the cultures of Pythium and Rhizoctonia, causing reduced emergence and increased post-emergence losses. Sclerotium bataticola caused the least emergence loss but post-emergence attack was relatively high. Pinus resinosa and Pseudotsuga taxifolia were equally most susceptible to both phases of the disease. Pinus ponderosa had the least emergence loss and Picea pungens the least post-emergence loss.

Nursery losses due to these fungi could be expected to be less severe than those thus experimentally induced; however, conditions under some circumstances might resemble those in the tests, in which event losses of similar

magnitude caused by normally saprophytic or moderately parasitic species of Fusarium could be expected.

BOTANICAL LABORATORY,

University of Pennsylvania.

PHILADELPHIA, PA.

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STUDIES IN THE FUSARIUM DAMPING-OFF OF CONIFERS. II. RELATION OF AGE OF HOST, pH, AND SOME NUTRITIONAL FACTORS TO THE PATHO GENICITY OF FUSARIUM¹

HOWARD TINT2,3

(Accepted for publication January 15, 1945)

The damping-off diseases of coniferous seedlings are usually epidemic in character. Their economic significance is due to the irregularity of the losses as well as to the severe reductions in nursery stands. In a single nursery, in one season, damage may be negligible or moderate; whereas, in the next growing period, losses may be severe. Adjacent seed beds may be attacked in highly varying degrees. Obviously, certain predisposing factors must operate, singly or in a particular combination, at any given time, to account for the variability in losses.

Under certain conditions many species of *Fusarium* cause severe reductions in emergence as well as high post-emergent mortality (36). The present investigation deals with variations in environmental conditions affecting this host-pathogen relationship.

GENERAL MATERIALS AND METHODS

The selection of hosts and damping-off fungi was based upon results from a previous experiment (36). The sources of seeds and of the Fusarium lines were described in detail, as well as standard methods of seed sterilization, selection of monosporous cultures of virulent strains of fungi, preparation of inoculum and glycerophosphate nutrient solutions for the hosts and fungi. A division of the damping-off losses into two categories, emergence loss and post-emergence damping-off has been described. The same division was followed in evaluating losses due to inoculations with fungi in the following tests. Where experimental procedure required occasional departures from these standard methods, the alternative treatments are described.

All seedlings were grown in a low-humidity greenhouse, thermostatically adjusted to maintain a daily mean temperature range of 68–75° F., in those seasons of the year when such control was possible.

Determinations of pH were made potentiometrically with quinhydrone and saturated calomel half-cells, and all solutions were adjusted in acidity with 0.1 N HCl and 0.1 N NaOH.

AGE OF HOST

Post-emergence losses from damping-off generally diminish with in-

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crease in age of coniferous seedlings (10, 11). Early losses have been associated with the tenderness of the young seedlings. With the development of woodiness in the hosts, ordinary damping-off losses became relatively negligible. Early post-emergence attacks, invariably fatal, have been included in the category of ordinary or normal damping-off losses. Those occurring after this initial period, and not necessarily fatal, have been described as root-rot. Emphasis of the present studies has been placed primarily on the former type, and an attempt was made to determine how long after emergence seedlings were subject to normal damping-off attack by Fusarium species.

Initial observations on the relation between age of host and susceptibility to attack were made upon seedlings of *Pinus resinosa* grown in quartz-sand

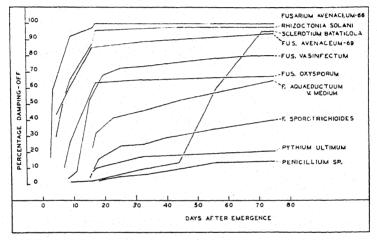


Fig. 1. Relation of time to post-emergence damping-off of *Pinus resinosa* seedlings inoculated in quartz-sand cultures with Fusarium and other standard fungus lines.

cultures, watered daily with the standard nutrient solution adjusted to pH 6. These were in 4-inch pots that had been inoculated with Fusarium or other fungus lines prior to the planting of fifty seeds in each. Periodic records were made of the losses that occurred after the emergence of the seedlings. The damping-off loss in each culture was recorded as the percentage of the total number of seedlings that had emerged up to that time.

The results are in figure 1, the curves being based upon the averages of duplicate series for each fungus line. On seedlings in contact with the more virulent fungi, effects of attack were visible soon after emergence, and maximum losses were reached within approximately three weeks. The effects of less virulent fungus lines did not become noticeable until later, although maximum losses developed before 30 days. For all of the lines there were slight increases in damping-off with time, following the initial surge of attack. Losses from Sclerotium bataticola were low for the first six weeks but became very pronounced thereafter, reaching an extremely high level at the termination of the experiment. This tendency for Sclerotium to be only

slightly effective in early attack, in comparison with Fusarium sp., has already been noted (36).

In order to verify that these variations in host-pathogen relationship were due to increasing resistance of the host with age, healthy seedlings of *Pinus resinosa* of varying ages were transplanted to pots of nutrient-watered sand, that had previously been inoculated with rice cultures of *Fusarium oxysporum* and incubated at room temperature for five days. Younger seedlings were taken from a sterile germinator, whereas older ones were grown in uninfected quartz-sand in the greenhouse. Each age group was tested in duplicate, at the rate of 40 seedlings per pot, and losses due to damping-off were recorded for 30 days after transplanting. Losses in the cultures, which were run simultaneously in the greenhouse, are given in figure 2.

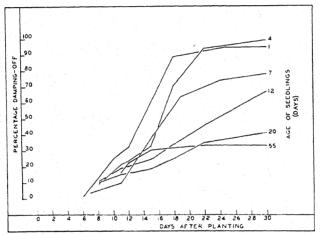


Fig. 2. Relation of age of seedlings of Pinus resinosa to rate and total extent of damping-off in quartz-sand cultures inoculated with Fusarium oxysporum.

Seedlings from 1 to 7 days old had the higher percentage of damping-off, and the reduction in levels of losses corresponded with the increasing age of the host. The time between initial exposure of the seedlings to the fungus and the first symptoms, varied from 6 days with the one-day-old seedlings to 11 days for the 55-day-old plants.

PH STUDIES

Soil reaction may affect forest-tree seedlings directly through the influence of hydrogen or hydroxyl ions, or indirectly by influencing the availability of nutrients, the physical condition of the soil, and the activity of parasitic and saprophytic soil-organisms. Conifers, in general, prefer an acid reaction for optimum seed germination (1, 4, 5, 6, 31, 37, 39) and for subsequent growth and development (6, 14, 38, 40). Species of Fusarium, in the laboratory, have a wide tolerance for variations in the reaction of substrates, ranging from approximately pH 2 to 11, but optimum growth has usually been observed on media of acid or nearly neutral reaction (17, 19, 20, 27).

Early observations by Gifford (9) revealed that an increase in the alkalinity of the soil by liming increased the amount of subsequent damping-off, whereas manuring treatments which tended to keep the soil reaction acid, reduced the damage. Anderson (3) observed that the application of wood ashes to seed beds before sowing increased damping-off of seedlings of *Pinus sylvestris* and *Picea sitchensis*. Hartley (10, 12) observed that damping-off in forest nurseries was not serious in soils with a pH lower than 6. In addition, soil acidification treatments have been used to control the disease (7, 10, 12, 18, 33, 35), although with only partial success in some localities.

These observations have to a large extent been confirmed by inoculation Roth's investigations (29) on *Picea excelsa* inoculated in soil cultures with Pythium deBaryanum and Rhizoctonia solani, revealed no serious losses at pH values below 5.5, maximum attack on the acid side of neutrality, and again a lessening of losses at pH values above 7. The last effect is at variance with general experience in the field, where heaviest losses occur at an approximate pH value of 8 (10). Inoculations of Douglas Fir and ponderosa pine with isolates of Pythium and Rhizoctonia by Jackson (16), gave similar results. In sand cultures, however, Jackson's results showed better agreement with field experience for the higher pH values, although somewhat more damping-off at the acid levels was obtained than under natural Ten Houten (35), from inoculation tests on Pinus nigra austriaca, in soils that varied in pH and in organic matter, concluded that the parasitism of Pythium deBaryanum, Rhizoctonia solani, and Fusarium oxysporum, was highly dependent on the reaction of the soil. In those soils in which the pH was 4.5 or lower, the damage caused by these fungi was not important.

A preliminary test of the relationship of pH and soil-sterilization to damping-off was performed in soil samples artificially adjusted into a pH series according to a method used by Roth (29). A soil was prepared from three parts of loam, two of sand, and one each of leaf-mold humus and acid peat (Canada). To half of this mixture one extra part of peat was added and to the other one part of humus, yielding respective pH values of 5.1 and By the addition of graded amounts of coarse sodium-calcium hydroxide (40 per cent NaOH, 57 per cent Ca(OH)₂, 2 per cent H₂O) to the more alkaline portion, samples with pH values of 6.9, 7.9, 8.3, and 8.6 were obtained. Similar treatment of the acid portion with sodium bisulphate (35-36.5 per cent H₂SO₄) yielded lots of pH 4.9 and 3.6. A mixture of the two original lots gave a sample of pH 5.6. In preliminary investigation, soil samples similarly adjusted maintained their new reactions rather closely for a long time if continually moistened with water adjusted to corresponding acidity levels. All acidity determinations, however, were made upon samples airdried in the laboratory for several days. This method of adjusting and maintaining the acidity of soil provided samples highly comparable in physical and basic chemical content, differing mainly in respect to the acidifying and alkalizing substances added. It permitted a more significant comparison of the effects of the reactions on the growth of the host and the dampingoff losses induced by inoculated fungi than would have been possible in a collection of soil samples from different localities.

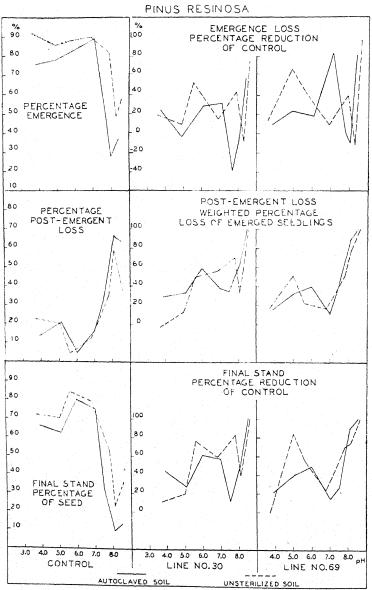


Fig. 3. Relation of pH to the growth and damping-off of *Pinus resinosa* in soil cultures inoculated with *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69). Results for inoculated series expressed in terms of corresponding control data.

Each pH lot was distributed into twelve 7-inch pots, and six of each group were autoclaved at 15 pounds for one hour on two successive days. The resultant pH values in the autoclaved samples were 3.8, 4.9, 6.1, 7.1, 7.7,

8.0, and 8.4. Duplicate pots were then inoculated with rice-mush cultures of *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69), sterile rice medium being added to the control series. After incubation for one week

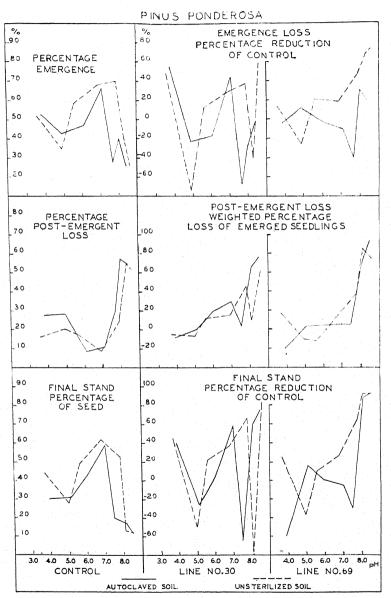


Fig. 4. Relation of pH to the growth and damping-off of Pinus ponderosa in soil cultures inoculated with Fusarium oxysporum (Line 30) and F. avenaceum (Line 69). Results for inoculated series expressed in terms of corresponding control data.

at room temperature, 50 seeds each of *Pinus resinosa*, *Pinus ponderosa*, and *Pinus sylvestris* were planted in each pot, and all series were placed in the greenhouse.

Emergence and post-emergence losses were tabulated during 5 months. At the termination of the experiment a survival count was made and the final pH of the various soils was measured. To evaluate the effects of the inocu-

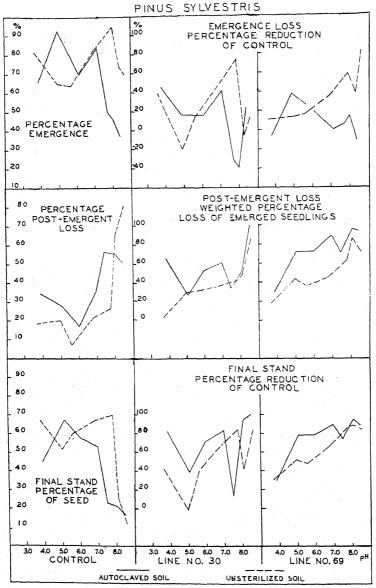


Fig. 5. Relation of pH to the growth and damping off of Pinus sylvestris in soil cultures inoculated with Fusarium oxysporum (Line 30) and F. avenaceum (Line 69). Results for inoculated series are expressed in terms of corresponding control data.

lations alone at the various pH levels, apart from those losses occurring as a result of the growth of the hosts in some instances in a physiologically unsuitable environment, seedling losses in inoculated pots were calculated in

terms of corresponding control data. The weighted results are given in figures 3, 4, and 5, which also include the original control results for purposes of comparison. Emergence data are plotted against initial pH values, and those for post-emergence loss and final stand against the average reactions that prevailed during the experiment.

The differences in pH, or their indirect effect, exerted a pronounced influence upon the relative emergence of the hosts, and also affected their ability to survive. Attempts to isolate organisms from seedlings that died in control pots showed that fungi could not be isolated from approximately half the seedlings, and that from infected seedlings, Fusarium isolations were no more numerous than other contaminants.

TABLE 1.—Final concentrations in parts per million of some constituents determined by dilute-acid extracts of soil samples adjusted and maintained in a pH range. A blank test indicates the substance is low beyond the sensitivity of the test reaction. The values for pH are average for the duration of the experiment

		pH values	for autocla	ved soil	s	
3.9	5.1	6.0	7.0	7.5	8.1	8.5
40	110	115	160	150	165	175
0	0	0	0	0	0	0
1-2	10	8	5	5-8	18	20
0	0	0	tr	1	tr	tr
5	6	2-3	0	tr	2	1
1-2	5	7-10	8	10	15-20	8
2	4	5-6	10	45	25	30
30	120	10	0	0	0	0
		pH values	for unsteril	ized soi	ls	
3.7	5.0	5.6	6.8	7.8	8.1	8.6
40	100	90	165	175	250	200
0	0	0	0	0	0	0
5-10	15	5	2	4-5	10-14	60
0	0	0	0	1	1	tr
3	2	\mathbf{tr}	0	1-2	1	1
1-2	3	8-10	12-16	10 - 12	12-14	12
2-3	5	5	8-10	15-16	45	55
50	100	0	tr		0	0
	40 0 1-2 0 5 1-2 2 30 3.7 40 0 5-10 0 3 1-2 2-3	40 110 0 0 1-2 10 0 0 5 6 1-2 5 2 4 30 120 3.7 5.0 40 100 0 0 5-10 15 0 0 3 2 1-2 3 2-3 5	3.9 5.1 6.0 40 110 115 0 0 0 0 1-2 10 8 0 0 0 0 5 6 2-3 1-2 5 7-10 2 4 5-6 30 120 10 PH values 3.7 5.0 5.6 40 100 90 0 0 0 5-10 15 5 0 0 0 3 2 tr 1-2 3 8-10 2-3 5 5	3.9 5.1 6.0 7.0 40 110 115 160 0 0 0 0 0 1-2 10 8 5 0 0 0 0 tr 5 6 2-3 0 1-2 5 7-10 8 2 4 5-6 10 30 120 10 0 PH values for unsteril 3.7 5.0 5.6 6.8 40 100 90 165 0 0 0 0 5-10 15 5 2 0 0 0 0 0 3 2 tr 0 0 1-2 3 8-10 12-16 2-3 5 5 8-10	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

At the several levels of acidity, there were pronounced differences in host-response with respect to emergence, survival, and damping-off to two Fusarium lines, both in autoclaved and unsterilized soils. However, there was no indication whether the effects were due to reaction differences directly, or to the indirect results of the chemical changes induced by the treatments of the soil samples. Analyses made at the termination of the experiments upon the uninoculated soil samples, according to the methods of Spurway (34), showed significant differences in the availability of certain constituents at the different levels of reaction, and between autoclaved and unheated samples. The results of these analyses are given in table 1. Certain of these differences could have been anticipated from the manner of soil treatment. Thus the increase in Ca availability with the rise in alkalinity

and of the SO₄ ion in the acid samples are understandable. The latter, however, gave a higher test at pH 5 than at the more acid levels to which it had been added in greater amounts. In view of similar variability of the other

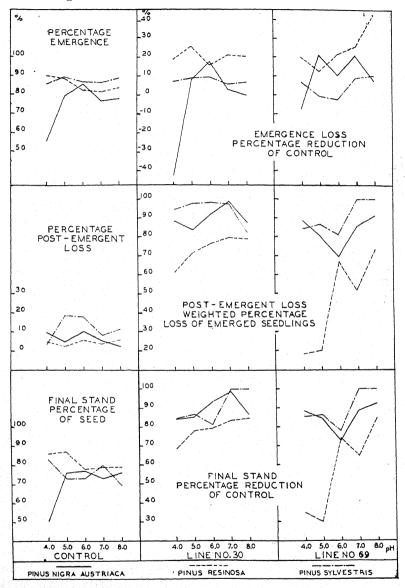


Fig. 6. Relation of pH to the growth and damping-off of three conifers in quartz-sand cultures inoculated with Fusarium oxysporum (Line 30) and F. avenaceum (Line 69). Results for inoculated series are expressed in terms of corresponding control data.

constituents, further experiments were necessary, in which the factors of reaction and nutrition could be independently controlled.

In order to establish the relationship of acidity alone to the development

and damping-off of conifers, inoculation tests were made in quartz-sand (Ottowa) substrates, watered by flooding daily with the basic nutrient solution adjusted in pH at one-unit intervals from 4 to 8 inclusive. The experiments were in duplicate on three hosts inoculated with two fungi, Fusarium oxysporum (Line 30) and F. avenaceum (Line 69). Fifty seeds of Pinus resinosa and 50 of Pinus sylvestris were sown separately in four-inch pots, and the same number of seeds of Pinus nigra austriaca in five-inch pots, all of which had been inoculated five days previously with rice cultures of the fungi. Control series received sterile rice mush. Emergence and losses were recorded for two months. The results, recalculated according to the method described, are summarized in figure 6.

Corresponding studies were made upon the effects of acidity variations

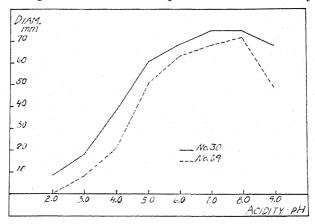


Fig. 7. Effect of acidity on the growth of Fusarium oxysporum (No. 30) and F. avenaceum (No. 69) on potato-dextrose agar.

upon the growth of the two Fusaria in Petri plates on potato-dextrose agar. The pH varied by one unit from 2.0 to 9.0, each treatment replicated five times. After incubation for six days at 28° C., two diameters at right angles were measured for each culture and averaged (Fig. 7).

NUTRITION

The concentration of the nutrient solution is an important factor in the growth and tolerance to environmental conditions of coniferous seedlings (8, 24, 25, 26, 41). A deficiency in any of the essential nutrients or an unbalanced ratio, as may well be induced by prevalent acidity conditions (13, 22, 28), influences the entire metabolic process, and as a consequence, may decrease the resistance of the seedlings to physiological and infectious diseases. Similarly, Fusarium species, as well as other soil organisms that may be potential damping-off agents, have specific nutritional requirements (17, 21, 27, 42), and the effects of alterations in concentration of nutrients, with changes in acidity, may conceivably be the underlying or a contributing factor to the various levels of pathogenicity of these organisms.

Nutritional aspects of the growth and damping-off of *Pinus resinosa* were studied in sand cultures, with excesses and deficiencies of calcium, phosphorus, nitrogen, magnesium, potassium, and sulphur in the standard nutrient solution. Since the original nutrient solution contained certain salts supplying two of these elements, in order to eliminate either one of them it was necessary to replace an equivalent concentration of the remaining constituent of the compound by sodium-salt or metallic chloride, depending upon whether it appeared as an anion or cation. An excess of any element was attained by trebling its molar concentration by the addition to the basic

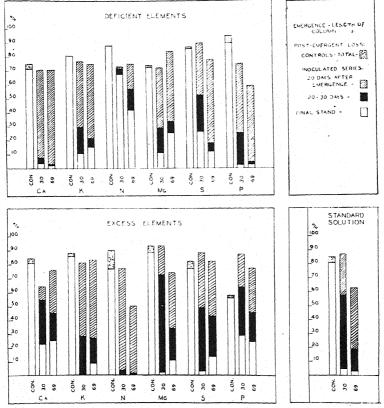


Fig. 8. Effects of deficiencies and excesses of various elements on emergence and damping-off of *Pinus resinosa* inoculated in quartz-sand cultures with *Fusarium oxysporum* (Line 30) and *F. avenaceum* (Line 69). Results are averages of duplicate trials.

solution of twice the original concentration of the particular ion as chloride or sodium-salt. In the case of phosphorus, additional treatment was necessary. Since originally this element was supplied in a glycerophosphate salt, used also as a source of carbon for the fungi, the removal of this ion from the solution necessitated the addition of an equivalent concentration of some carbon source, in this case dextrose. Conversely, an excess of phosphorus was attained by the use of Na₂HPO₄, in order not to increase the basic carbon content of the solutions. All solutions before use were adjusted to a final pH of 6.

TABLE 2.—Results of the growth of Pinus resinosa seedlings in quartz-sand cultures deficient in certain elements. Duration of experiment 30 days following emergence. Daily mean temperature range 68-75° F.

Element	No. of seed-	ining i and			Ratio of green	
lings	Roots	Hypo- cotyls	Green	Dry	to dry weight	
Ca	23	13.7	43.9	43.4	4.6	9.4
K	21	11.2	39.3	33.9	5.5	$6.\overline{2}$
. N	30	21.5	42.4	45.6	7.1	6.4
Mg	11	10.9	35.3	43.5	5.3	8.2
$egin{array}{c} \mathbf{M}\mathbf{g} \\ \mathbf{S} \end{array}$	30	12.6	39.9	44.0	4.7	9.4
P	27	12.7	43.3	44.6	3.9	11.4
\mathbf{Full}	30	13.4	43.7	44.0	6.0	7.3

The inoculation procedure was modified to prevent any great change in the concentrations of the various nutrients which might result from the addition of rice to the substrate. Fusarium oxysporum and F. avenaceum were grown on 50 cc. of an aqueous extract of boiled rice, to which had been added 0.5 per cent dextrose. After incubation for three weeks at 28° C., the mycelial mats were removed, thoroughly washed with distilled water, and then ground with sterile sand in a mortar. This was distributed as inoculum, at the approximate rate of one-half mat per pot, into four-inch pots of autoclaved sand, which were subsequently saturated with the various nutrient solutions. The pot cultures were incubated for five days at room temperature and 50 seeds of Pinus resinosa were then sown in each. Duplicate series for both fungi and controls were maintained for 30 days following general emergence of the seedlings, the pots being watered daily by flooding with the corresponding solutions.

The results are given in figure 8. At the termination of the experiment, the seedlings were removed from the substrate and certain growth characteristics measured. These have been summarized in tables 2 and 3.

The responses of Fusarium oxysporum to nutritional conditions were tested in 5 replicates on agar substrates (1.5 per cent) in Petri dishes, pre-

TABLE 3.—Results of the growth of Pinus resinosa seedlings in quartz-sand cultures with excesses of certain elements. Duration of experiment 30 days following emergence. Daily mean temperature range $68-75^{\circ}$ F.

$egin{array}{ll} ext{No. of} \ ext{Element} & ext{seed}. \end{array}$						Average milli	Ratio of green	
Liemei	1t	lings		Roots	Hypo- cotyls	Green	Dry	to dry weight
 Ca		30		20.5	38.4	41.2	5.7	7.2
K		30		19.5	39.6	43.0	5.7	7.5
N		22		18.6	38.8	42.8	5.9	7.3
Mg		36		20.6	38.9	44.7	5.5	8.1
Mg S		35		19.2	39.1	41.8	5.6	7.5
P		24		18.2	37.7	43.5	5.8	7.5
Full		32		20.1	39.9	46.0	5.6	8.2

pared from solutions containing corresponding excesses and deficiencies of elements, and adjusted to yield pH 6 after sterilization. The average diameter of each colony was determined after incubation for 6 days at 28° C. The results, given in table 4, indicate significant differences in the growth of the fungus under the several conditions. Removal of a single element in each case reduced the growth in comparison to that on the standard solution.

TABLE 4.—Results of the growth of Fusarium oxysporum in synthetic-nutrient agar containing deficiencies and excesses of certain elements

	701 4	Average diam	eters of colonies in 1	nm.
	Element	Deficiency	Excess	
	Ca	74.7	84.5	
	K	69.0	81.4	
	N	65.7	86.4	
	Mg	72.6	80.1	
	Š	69.4	82.6	
	P	67.6	78.5	
	Full		82.4	

DISCUSSION

Inoculations in sand cultures, adjusted over a pH range, with two species of Fusarium upon three coniferous hosts, revealed, on the average, a direct correlation between reaction and damping-off. Although variance was evident in the reactions of the hosts among themselves and between fungus treatments, a definite trend nevertheless was indicated for losses to become greater with decreasing acidity. The differences in parasitism at the various pH levels were most likely dependent upon the direct effect of the reaction of the media on the growth of the pathogens. With the single exception of the reduction in emergence of Pinus nigra austriaca on the most acid medium, different levels of reaction apparently had no significant effects upon the hosts, measured in terms of emergence and survival. In contrast, however, both fungi were directly affected by the changes in acidity, and their relative growth at the various levels was directly correlated with the losses induced. Jackson (16) also has interpreted the lower losses under more acid conditions with inoculations of Pythium and Rhizoctonia on conifers in sand and liquid cultures, in terms of a direct effect of the reaction on the growth of the parasites.

The results of soil tests of reaction differences indicated a far greater variability for damping-off as well as for emergence and survival of hosts in uninoculated controls. In both autoclaved and unsterilized soils, losses were generally negligible or moderate at the extreme acid end of the range. Maxima were reached approaching neutrality and the losses dropped sharply in most cases at pH 7.5 and 8.0, to be followed later by severe or complete losses at the most alkaline values. Roth (29), reporting results from a study of the effect of pH on the damping-off of Norway spruce (*Picea excelsa* Link) grown under somewhat similar soil conditions, found losses caused by *Cor*-

ticium vagum and Pythium deBaryanum definitely increased from pH 3.8 to maxima between 6.5 and 7.0 and then decreased at pH 8.0. These facts agree to some extent with the present results, although they were not extended so far with regard to more alkaline substrates.

The variance between the results derived from sand- and soil-cultures undoubtedly resulted from the additional chemical variability in the latter. Deficiencies and excesses of certain elements which varied in the soil cultures, tested under controlled conditions of reaction in sand-culture, were associated with losses from damping-off. It was frequently possible to correlate the magnitude of the attack with the simultaneous effects of these nutritional conditions upon the host and pathogen. Varying nitrogen gave the most pronounced differences in this respect. Where this element was deficient the growth of the fungus was least, whereas the host had optimum root development as well as a relatively low degree of succulence, expressed as ratio of green to dry weight. There was little loss from damping-off. An excess of nitrogen, although mildly affecting the host by a slight reduction of root length, unquestionably stimulated fungus growth sufficiently to account for the rapidity and severity of its subsequent attack. Hartley (10) has likewise expressed the belief, based on general nursery experience, that conifers grown on a soil rich in nitrogen are especially susceptible to damping-off.

Illick and Augenbaugh (15), in studies on Pinus rigida, stated that an excess of lime in the soil creates conditions which are most favorable for the activities of damping-off fungi, although no mention is made of any direct effect of excess lime on the seedlings. In the present experiments, a deficiency of calcium, inducing a high degree of succulence of the seedling, combined with the best fungus growth of the entire deficiency series, resulted in early and heavy attack. On the other hand, an excess of calcium yielded a hardened seedling with good root development, by virtue of which the host apparently was able to withstand rather good growth of the fungus. suggests that the high mortality associated with alkaline calcareous soils is due to the reaction directly rather than to the high calcium content. In fact, Albrecht and Jenny (2), in studies of the damping-off of soy bean seedlings, concluded that within a wide range of pH (3.8-8.0), heavy damping-off occurred only when the calcium supply was low, but none took place when the supply of calcium was high. Chapman (6) indicated that pH and soluble-calcium content may independently influence survival of shortleaf pine (Pinus echinata Mill.); the latter factor, however, restricting survival with concentrations of 500 parts per million or higher. Howell (14) observed that calcium is not deleterious to seedlings of Pinus ponderosa, and suggested that the fact that the ion is not assimilated by the plant in alkaline soils may be important. In the soil experiment of the present investigations, the calcium content was increased simultaneously with the alkalinity of the medium, and the higher losses that resulted may have been due to the masking of the beneficial effect of calcium, present in less than toxic concentrations, by the alkaline environment.

The concentration of phosphorus likewise markedly affected the growth of the host as well as the extent to which it damped-off. Under a deficiency of this element, the seedlings were the most succulent of the entire lot and were extremely susceptible to attack in spite of the fact that the fungus grew rather poorly. With an excess of phosphorus, the seedlings became moderately hard and the fungus growth was poorest of the entire excess series, both factors combining to yield a relatively lower level of attack. Availability of this element appeared to be a factor related to the reduction of losses in the soil experiments on the alkaline side of neutrality. These low points generally occurred at pH 7.5 in the autoclaved soils and at 8.1 in the unsterilized lots. Both points coincided with a high content of available phosphorus. The availability of this element has been associated with buffering of plant cytoplasm and cell sap (6), its reduction in the substrate being associated with a lower degree of buffer capacity for alkali. Addition of superphosphate to an acid nursery soil in which seedlings were developing, somewhat increased the ability of the plants to withstand a wide range of environmental conditions (14).

Damping-off of the seedlings, under the influence of variations in the concentrations of the other elements tested, was less consistent than were the effects of the conditions upon the hosts. In general the reduction of losses where the test elements were deficient, as compared with the control, appeared to be related to the corresponding reduction in growth of the fungi. In those media in which all the elements were present in their basal concentrations, the growth of the colonies appeared little influenced by specific excesses, and the losses were comparable with those in the standard solution. Young and Bennett (42) have shown that the proper balance of the inorganic constituents in the solution is essential for the maximum growth of Fusarium oxysporum and other fungi. In the present experiments, this balance was more affected by the removal of an element from the nutrient solution than by an increase in its concentration.

The chemical factors were connected with steam sterilization of soil. Heating the soil produces pronounced chemical and biological changes (10, 23, 30, 32), which must be considered in evaluating the treatment to control damping-off. Lindfors (19) concluded that steam-sterilization of soil created conditions favorable for reinfection by Fusarium. Ten Houten (35) found a similar increase in attack with *Rhizoctonia solani* on sterilized soil, and attributed the higher damage to the removal by the heating process of organisms antagonistic to the pathogen. A similar view was taken by Hartley (10). Scheffer (30) observed that a heat treatment of soil was not detrimental to subsequent growth of several conifers; in fact, plants grown in the heated soil developed somewhat better roots and crowns. However, as indicated by the results of the present experiments as well as other observations (23, 32), steam sterilization may change the reaction of a soil as well as its basic nutritional equilibrium. Some of these factors may operate independently, or in combination, upon host and pathogen alike to affect directly the

amount of disease. Thus the effectiveness of the treatment depends upon whether the conditions favor or oppose the relationship. The present results have indicated that the treatment increased subsequent losses as often as it reduced them, with no apparent consistency, over the entire range of soil samples. Similar conditions in the field undoubtedly account for the variability and contradiction of the reports on the usefulness of this method.

SUMMARY

In seedlings of *Pinus resinosa* grown in quartz-sand culture and inoculated with Fusaria and other fungus lines, losses due to more virulent fungi appeared relatively early and reached their maximum levels within approximately three weeks following emergence, whereas those due to less virulent lines were delayed, although generally reaching maximum levels before thirty days. Losses from *Sclerotium bataticola*, a comparison line, were low for the first six weeks, but increased thereafter and attained a high level before seventy-five days.

Tests with seedlings of various ages, inoculated with *Fusarium oxy-sporum* under identical conditions, demonstrated that resistance to invasion increased directly with increasing age of *Pinus resinosa*.

The relation of acidity to damping-off was tested in cultures of a single soil the pH of which was varied artificially. In samples, subdivided to include autoclaved and unsterilized lots, emergence reductions of three coniferous species due to inoculations with Fusarium oxysporum and F. avenaceum were generally low or moderate at pH values in the acid range and high above pH 8. In some cases emergence in inoculated pots was greater than in corresponding controls at pH 7.5 in autoclaved samples and at pH 4.9 and 8.3 in unsterilized soils. Post-emergence damping-off was low in more acid samples, reached a maximum on the acid side of neutrality, fell off sharply in the autoclaved soil at pH 7.5 and in the unsterilized soil at pH 8.1, and then became uniformly high in the most alkaline substrates. Differences were not consistent at the various levels of acidity from autoclaved and unsterilized soil samples. The chemical variability in the soil samples, which resulted from the induction of reaction differences and from steam-sterilization is discussed.

In quartz-sand cultures of varying pH, losses of three conifers inoculated with the same fungi were generally high at all levels of acidity, although there was some tendency for increased damping-off with increasing alkalinity.

Tests in quartz-sand cultures on the effects of variations in the concentration of certain constituents of the nutrient solution, under uniform conditions of reaction, demonstrated that the influence of nutritional factors on damping-off could be correlated with their simultaneous effects upon the host and pathogen. The magnitude of the loss was associated with the growth of the pathogen and the degree of susceptibility of the host, measured in terms of its succulence. A deficiency of nitrogen gave relatively negligible

losses, whereas an excess caused early and heavy attack. Deficiencies of calcium and phosphorus resulted in high damping-off losses, which were considerably reduced when these elements were present as excesses. When potassium, sulphur, and magnesium were deficient, final stands were somewhat higher than under excesses of these elements, the latter resembling controls grown and inoculated in standard solutions.

BOTANICAL LABORATORY,

University of Pennsylvania, PHILADELPHIA, PA.

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THE EFFECT OF ARTIFICIAL LIGHT ON GERMINATION OF UREDIOSPORES OF PHRAGMIDIUM MUCRONATUM (FR.) SCHLECHT.¹

VINCENT W. COCHRANE

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INTRODUCTION

Dillon Weston (2, 3, 4) found that the germination of urediospores of *Puccinia graminis tritici* is inhibited by intense light of the visible range. Separation of white light into its components demonstrated that the inhibitory effect is a property of the longer rays (red, orange, yellow) of the spectrum; germination was not affected by blue or green light.

Stock (10) reports no effect of strong artificial light on the germination of urediospores of *Puccinia triticina*, *P. dispersa*, and *P. coronata*, except a slight delay in the last two species in the time of movement of the spore protoplast into the germ tube. In the case of *P. graminis* the germination was somewhat delayed by strong light, but the eventual germination was as high in the light as in the dark.

A similar retarding influence of diffuse sunlight has been found in the case of *Puccinia glumarum tritici* (11).

Negative phototropism of germ-tube growth has been observed in the case of species of *Puccinia* (6, 7).

Studies on other rusts (5, 8) have failed to establish any effect of light on urediospore germination.

The data here presented were obtained during a general investigation of the physiology of spore germination in *Phragmidium mucronatum* (Fr.) Schlecht., cause of the common leaf rust of cultivated roses.

MATERIALS AND METHODS

A single-urediospore line of the pathogen was propagated on the variety Briarcliff and used in all experiments. This clone was isolated originally from infections on leaves of the variety Christopher Stone received from California.²

Mature urediospores were brushed from the sori into a beaker, then transferred to a small "puff-duster" made of glass tubing. The spores were blown from this onto the surface of 2 per cent water agar contained in 7-cm. Petri dishes. Methods of collection and germination are more fully described elsewhere (1).

The experiments were made in a thermostatically controlled constant-temperature room. The light source was a 500-watt "Mazda" CX bulb.

¹ Excerpt from a thesis presented April, 1944, to the faculty of the Graduate School of Cornell University, in partial fulfillment of the requirements for the degree of doctor of philosophy.

² The writer is indebted for the original rust material to Dr. H. Earl Thomas, Dept.

of Plant Pathology, University of California, Berkeley, Cal.

Interposed between the light and the germination surface were a layer of water, a thin sheet of window glass, and a Corning "AKLO" No. 395 filter. This filter was selected so that, in combination with the filtering action of the water layer, practically all of the infra-red rays were screened out. Various light intensities were obtained by placing the germination dishes at appropriate distances from the light source. Light intensity was measured with a Weston Model 603 light meter equipped with a "Viscor" filter.

The significance of observed differences in germination was determined by means of the Chi-square test (9).

RESULTS

A preliminary experiment demonstrated a marked inhibition of germination at a light intensity of 3,000 foot-candles and a temperature of 22–24° C. Germination was 79.2 per cent in controls held in the dark at this temperature, 17.1 per cent in lots exposed to the light.

TABLE 1.—The influence of various intensities of artificial light on urediospore germination. Checks B, D, and F wrapped in black paper and lead foil; checks G, H, and I in dark chambers

nt	Light intensity,	Temperature,	Per cent germinationa at				
Series	foot-candles	•C.	3 hr.	6 hr.	24 hr.		
A B	1250 0 (check)	19.5–20.0 19.5–21.5	7.0** 85.0	28.6** 92.9	42.2** 93.7		
\mathbf{C}	200 0 (check)	$\substack{19.0-20.0\\19.0-20.0}$	80.4** 87.0	$91.8 \\ 91.2$	93.0 92.3		
E F	2.4 0 (check)	19.0 19.0	88.2 87.8	$92.2 \\ 91.6$	$90.8 \\ 92.1$		
G H I	0 (check) 0 (check) 0 (check)	$18.0 \\ 21.0 \\ 25.0$	87.4 86.4 27.0	92.2 92.6 62.6	93.9 92.8 62.4		

^a Three- and six-hour counts based on 2 replicate samples of 250 spores each; 24-hour count based on 4 samples of 250 spores each.

Double asterisk indicates that germination differs significantly (99.1) from that of corresponding check.

A comprehensive experiment to measure the effect of various light intensities on the rate of germination and its final value is summarized in table 1. Since the optimum temperature for urediospore germination in *Phragmidium mucronatum* is 15–21° C. (1), the highest light intensity tested was one that did not raise the temperature above 21° C.

Three sets of 4 Petri dishes were sown and placed in the lighted chamber so as to receive light of three different intensities—1,250, 200, and 2.4 foot-candles. Similar plates wrapped in black paper and lead foil were placed adjacent to the exposed plates to serve as checks. Temperature was recorded periodically from wrapped and unwrapped thermometers at the 3 stations. Wrapped thermometers were enclosed in black paper and laid horizontally beside the germination plates. Lead foil was not used in wrapping these thermometers since it seemed that the heat-trapping effect of the black paper

wrapping would ensure that the maximum temperature would be reached. The temperature of the unwrapped plates was arrived at by periodic readings of unwrapped thermometers resting horizontally on the table beside the plates.

A further series of checks (Table 1, G, H, and I) consisted of replicate plates sown with spores and held in the dark in temperature chambers at 18°, 21°, and 25° C. respectively.

The injurious effect of light at 1,250 foot-candles intensity is clearly demonstrated (A). Both the rate of germination and the final value reached are significantly lower than the control (B). Chi-square comparisons show that only in the plates exposed to light of 1,250 foot-candles intensity was the attainment of the final level of germination delayed beyond 6 hours; in all other plates the germination at 6 hours was as high as that at 24 hours.

Light at an intensity of 200 foot-candles (C) exerted a slight delaying action on spore germination. This effect was evident at 3 hours from sowing, not at 6 or at 24 hours, *i.e.*, there was no permanent inhibition.

At the lowest intensity tested, 2.4 foot-candles, light had no effect on germination (E). The plates of this treatment were placed laterally in relation to the light source, but no evidence of phototropism of germ-tube growth could be seen. Other plates (A, C) that were illuminated were directly under the light source and therefore offer no data on phototropism.

None of these effects can be explained on the basis of temperature, with the necessary reservation that temperatures inside of the spore cannot be measured and may exceed air temperature. In all cases the temperatures of the wrapped check plates (B, D, F, in table 1) were as high as those of the lighted plates. Furthermore, germination in the dark at 25° C. (I) was significantly higher at all times than germination on plates at 19.5–20.5° C. and high light intensity (A).

Effects of ultraviolet rays may be excluded, inasmuch as the light passed through a layer of window glass and the glass Petri-dish lid before reaching the spores.

Spores that had been exposed for 24 hours to light of high intensity (Table 1, A) were removed to a dark chamber at 18° C. After 24 hours under these conditions, germination had increased from 42.2 to 54.5 per cent. In other words, 21 per cent of the spores that had failed to germinate during the exposure to light were still viable at the end of that period, 79 per cent were dead.

Dry spores in thin-walled glass vials were exposed to light of 1,250 foot-candles intensity for 24 hours at 21° C. Germination of these spores at the end of this period was not significantly different from that of control spores held in the dark.

DISCUSSION AND SUMMARY

Under strong artificial light (1,250 foot-candles) both final germination and rate of germination of urediospores of *Phragmidium mucronatum* on

water agar were depressed. For the great majority of the illuminated spores this phenomenon was not merely inhibition but was a lethal effect. A test of germination in the dark of previously illuminated spores showed that only 21 per cent of the ungerminated spores were germinable after 24 hours exposure, the larger proportion of the spores having been killed by the exposure to light.

It is, however, possible that the apparent lethal effect of strong light is only indirect. Spores prevented from germination, but not otherwise injured, by the light may have lost their viability as the result of the leaching of soluble cell constituents into the agar medium. Unpublished experimental investigations demonstrated that spores on agar lose their viability rapidly if prevented from germinating by low temperatures. In one such case storage of spores for 24 hours on agar at 3° C. resulted in a decline in viability from 91.1 to 25.4 per cent.

The subsequent germinability of dry spores was not affected by an exposure of 24 hours duration to light of an intensity of 1,250 foot-candles.

For the sake of comparison with natural conditions it may be noted that light intensity at midday in the summer commonly reaches the value of 10,000 to 12,000 foot-candles.

Spores germinating on agar under an illumination of 200 foot-candles intensity were slightly delayed in germination relative to unlighted controls at the same temperature. The inhibitory effect of light of this intensity was evident only during the first 3 hours of the germination period.

Light of low intensity (2.4 foot-candles) had no effect on urediospore germination. There was no evidence of phototropism of germ tube growth when the germinating spores were illumined laterally by light of this intensity.

DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY,

ITHACA, NEW YORK.

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LATE BLIGHT FORECASTING SERVICE¹

WAR SERVICE COMMITTEE OF THE UPPER MISSISSIPPI
VALLEY PLANT PATHOLOGISTS
1. E. MELHUS, CHAIRMAN, SUB-COMMITTEE ON LATE BLIGHT

In 1943 late blight forecasting was undertaken as a war service by a subcommittee of the "War Service Committee of the Upper Mississippi Valley" Plant Pathologists. The estimated loss of 25,000,000 bushels of potatoes in 1942 called for prompt and coordinate action of each and every State and Province in the region. The project received its initial impetus following the reading of a paper entitled "The Late Blight Outbreak of 1942 and a Proposed Forecasting Service" before the Iowa Vegetable Growers Association meeting in the fall of 1942. As a result of the proposal outlined for Iowa, R. J. Haskell called together representatives of different state potato certification agencies present to consider preparations for combatting late blight on a regional basis in 1943. Those present were M. B. Moore and A. G. Tolaas of Minnesota, R. D. Butcher of North Dakota, H. M. Darling of Wisconsin, E. P. Barrios of South Dakota, John McLean of Colorado, G. C. Kent, E. L. Waldee, and I. E. Melhus of Iowa.

The problem of being prepared to combat late blight was considered from the standpoint of forecasting, spraying, and availability of spray materials. Everyone present was keenly interested in blight control and believed much would be gained through a forecasting service. The several inspection agencies present were prepared to render all possible service.

Those present unanimously endorsed the following statement: "Because of the likelihood of destructive epiphytotic development of late blight of potatoes in 1943, due to the abundant and widespread distribution of inoculum in potato seed stocks, it is urged that Extension Pathologists and others cooperate to the fullest extent in providing for a blight development intelligence service and in preparing growers for quick and effective spraying to prevent a repetition of the great losses of 1942."

The sub-committee was organized with representatives from each State and the two Prairie Provinces of Canada in the region to report the weather and the state of development of the late blight pathogen in their respective States or Provinces. These data were sent to the chairman at Ames, Iowa, as they became available. He, with the assistance of Mr. Hans Frey in 1943 and Dr. Edgar F. Vestal in 1944, tabulated the data and issued the reports and forecasts.

TEMPERATURE, RAINFALL, AND LATE BLIGHT PREVALANCE AND DESTRUCTIVENESS

In figure 1 are recorded the temperature and rainfall from 1941 to 1944 inclusive for Illinois, Indiana, Iowa, Michigan, Nebraska, North Dakota,

¹ Journal paper No. J-1296 of the Iowa Agricultural Experiment Station, Ames, Iowa, Poject No. 450.

² W. E. Brentzel, W. F. Buchholtz, J. H. Craigie, Carl J. Eide, W. J. Henderson, James H. Jensen, G. C. Kent, William A. Kreutzer, R. H. Larson, L. R. Tehon, M. B. Linn, J. E. Livingston, J. H. Muncie, R. C. Rose, R. W. Samson, Paul Tilford, T. C. Vanterpool, I. E. Melhus, Chairman.

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South Dakota, Minnesota, and Wisconsin. Temperature and rainfall data for Manitoba are not at hand, through no fault of the pathologists of that Province, but a report for the Province will be included. The amount of late blight was so small in Missouri and Kansas and the Province of Saskatchewan that no attempt was made to correlate the temperature and rainfall with the late blight occurrence.

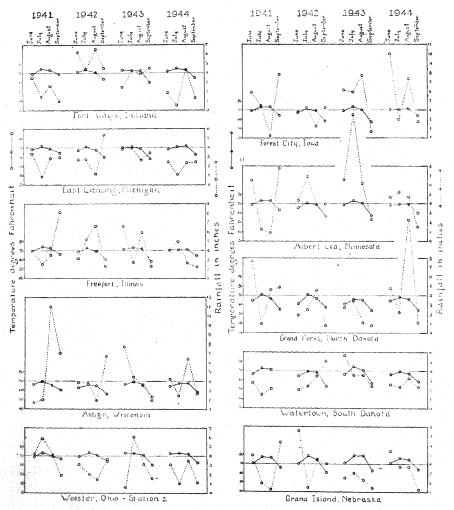


Fig. 1. Monthly mean temperature and rainfall in nine States, 1941-1944.

At the outset it should be pointed out that the available data from the respective States were limited; the weather records from only one station were used. Sometimes the chosen station was not in the potato growing region. In other cases the disease survey was very limited because no one was available to make the observations and there was often little uniformity in the records. On the other hand, in attempting to learn whether a fore-

casting service could be established in a large region, the lack of extensive data may well have been an advantage in testing the feasibility of the undertaking.

In any event, imperfect as the records were, and granting that some mistakes may have been made, it is significant to know that by using the influence of temperature and rainfall on the development of the organism, its destructiveness can be predicted.

The line at 70° F. in figure 1 designates the temperature above which growing conditions were not highly favorable for the late blight organism in its initial stages of development. In no case has late blight become seriously destructive where the mean temperature was above 70° in June and July. The 70° point was of much assistance in formulating a basis for predictions as the reports arrived from the committee members.

For an outbreak of late blight to develop, three conditions must be fulfilled: first, there must be a source of inoculum, such as infected seed pieces, cull piles, volunteer potatoes, etc., second, the temperature and moisture conditions must be such that the pathogen can grow and fruit, and, third, there must be abundant susceptible host tissue readily available. The first and third conditions prevail almost every season, but the second is more rare. The organism survives in seed in storage, is carried to the field and planted, or it may survive in cull piles or dump piles near villages and farms where potato houses are located.

The Upper Mississippi Valley States rather readily fall into at least three groups with respect to temperature. The eastern section consisting of Ohio, Indiana, and Illinois, represented by Wooster (Station 2), Fort Wayne, and Freeport, respectively, form one group. The temperature in each of these States for the four years 1941–1944 was in general unfavorable for late blight. The 1942 season was probably the most favorable, and the 1944 season the least. These three States also reported the lowest losses.

Michigan, Wisconsin, Minnesota, North Dakota, South Dakota, and Iowa form another group. In these States the temperatures for the points chosen were much more favorable for the late blight organism. The favorable conditions were especially evident in 1942, 1943, and 1944.

Nebraska and Colorado fall into a third class because of the normally low rainfall and the use of irrigation for growing the majority of the crop. In Nebraska, it was only the early crop in the south central part of the State that was injured. Late blight was not found on the foliage in Colorado.

Probably the most significant fact apparent in the study of the data from each State is that in spite of much favorable weather in 1943 and 1944 there was a general reduction in losses suffered by the potato crop in the region.

The forecasting that was done throughout the season was based upon weekly weather reports but it will be observed that for the temperature and rainfall graphs in figure 1 and the data in table 2 monthly averages have been used. The use of weekly temperature and rainfall averages would have been desirable but would have been inconsistent with the amount of space which could be allotted for that purpose.

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^bIn Indiana losses were severe on June plantings in 1943; in North Dakota there was 1 per cent loss on vines and 5 per cent loss on tubers a Late blight found at Marietta, Ohio, in 1944; ? indicates uncertain date or no information.

The Bed River Valley of Minnesota sales were greater in 1944 than in 1943.

In the Bed River Valley of Minnesota sales were greater in 1944 than in 1943.

An asterisk indicates power driven machines. In Wisconsin the first number refers to machines used by large growers. In Minnesota the large growers had dusters and six airplane dusters operated in the Red River Valley in 1944.

In Colorado 20 per cent of the growers spray and 5 per cent dust.

The data in table 2 on the mean monthly temperatures for the four years show that the temperatures for June and September tend to be below 70° F. and the temperature was higher in June than in September. July was some-

TABLE 2.—Mean monthly temperatures for 1941 to 1944 inclusive, for June, July, August, and September for eleven states

State	Station	Year	Mean monthly temperature (°F.)			
State			June	July	August	September
Ohio	Wooster 2	1941	69.6	73.5	69.5	67.4
		1942	69.8	73.6	70.6	64.4
		1943	73.6	73.2	71.0	60.8
		1944	72.8	73.1	72.4	63.1
Indiana	Fort Wayne	1941	69.0	74.25	73.25	68.6
		1942	71.0	73.75	70.75	63.4
		1943	73.0	72.75	72.0	59.5
	-	1944	71.5	74.5	73.6	65.0
Illinois	Freeport	1941	69.6	74.0	72.6	66.4
		$1942 \\ 1943$	$68.8 \\ 71.6$	$72.6 \\ 73.7$	$69.9 \\ 72.0$	60.8 58.3
		$1945 \\ 1944$	71.0 71.4	71.0	72.0	64.6
35.11	774 T					
Michigan	East Lansing	$1941 \\ 1942$	$\begin{array}{c} 68.1 \\ 67.0 \end{array}$	$71.1 \\ 70.2$	$68.4 \\ 67.8$	$64.4 \\ 59.4$
		1943	69.6	70.4	68.6	57.5
		1944	68.4	70.4	71.0	62.1
Wisconsin	Antigo	1941	66.1	69.7	66.0	60.8
Wisconsin	Antigo	1942	63.0	66.6	65.7	56.3
		1943	66.7	69.8	67.4	53.8
		1944	65.6	68.0	68.4	59.2
Minnesota	Albert Lea	1941	69.0	73.8	73.3	63.6
THE SOLE	2210010 2200	1942	66.0	70.7	69.4	56.9
		1943	69.1	73.4	70.6	56.8
		1944	68.5	69.1	69.8	60.6
North Dakota	Grand Forks	1941	64.8	71.4	66.8	56.0
		1942	61.8	71.8	67.1	57.6
		1943	61.1	66.4	65.6	54.0
		1944	64.2	68.2	66.4	56.2
South Dakota	Watertown	1941	66.4	72.7	71.7	
		1942	64.4	69.5	68.5	53.9
		1943	66.3	74.3	70.5	56.3
		1944	66.1	69.6	67.7	58.3
Iowa	Forest City	1941	69.0	73.4	73.4	64.6
		$1942 \\ 1943$	67.0 69.5	$\begin{array}{c} 71.1 \\ 73.4 \end{array}$	69.8 70.8	58.2 57.4
		$1945 \\ 1944$	70.75	$70.4 \\ 70.75$	$70.8 \\ 71.2$	64.0
37.7	Grand Island	1941				
Nebraska	Grand Island	$\begin{array}{c} 1941 \\ 1942 \end{array}$	$70.0 \\ 70.4$	$\begin{array}{c} 77.6 \\ 78.6 \end{array}$	$76.6 \\ 74.4$	66.0
		1942	70.4	79.0	79.0	61.4 63.6
		1944	71.9	76.5	75.6	65.6
Colorado	Greeley	1941	65.4	72.0	70.0	58.6
Colorado	Greeney	$1941 \\ 1942$	65.7	73.0	70.2	60.0
		1943	65.3	75.1	74.2	61.2
		1944	66.6	71.8	72.8	61.7

what warmer than August. When the temperature conditions in June and July were favorable for the development of the pathogen, 70° or below, the disease became prevalent. Its subsequent destructiveness is influenced favorably or adversely by the temperature in August. September was al-

ways sufficiently cool to permit the organism to develop freely, providing ample susceptible foliage existed. In other words, the conditions in June and July govern the initial stages of the pathogen and those in August the final stages.

DEVELOPMENT OF THE PATHOGEN AND CONTROL PRACTICES

According to the data recorded in table 1, the late blight pathogen most often made its first appearance in June and July except in North Dakota, Illinois, Indiana, and Michigan where it was most often first seen in August. In all States it was recorded most prevalent in August or September except in Michigan where in 1944 it did not become most prevalent until October. The late blight disease was least destructive in 1941 and most destructive in 1942. In 1942 the estimated loss was about 25,586,000 bushels. In 1941 the only State that reported more than a trace of late blight was Wisconsin which recorded an estimated 8 per cent. In 1943 nine States reported an estimated loss of about 6,164,000 bushels. Just how much the spraying and dusting program reduced the loss is not apparent from the data in hand, but the sharp increase that took place in control practices doubtless had a strong retarding effect on the development of the pathogen.

The losses in 1944 ranged from a trace to 10 to 15 per cent. The only States that experienced important losses were Wisconsin with 12 per cent, Minnesota with 10 to 15 per cent, and North Dakota with one per cent foliage loss and 5 per cent tuber rot. It is impossible to convert these losses for 1944 into bushels because yield data are not at hand, but it is probable that the loss from late blight over the region in 1944 was considerably less than in 1942.

The most significant result apparent in the data shown in table 1 was the increase in control practices. In most of these States there was a sharp increase when 1941 and 1942 are contrasted with 1943 and 1944. The number of dusters and sprayers doubled (600 to 1,200) in four years in North Dakota. The increase in machines was sharply upward also in several of the other States, as Wisconsin, Minnesota, Michigan, Iowa, and Nebraska. In Minnesota, six airplane dusters were in operation in 1944.

The amount of copper sold in different forms seemed to increase from year to year but this probably is a less reliable criterion of the control effort than the number of sprayers and dusters. In most cases it was difficult to obtain complete information relating to the amount of fungicide used.

This vigorous effort in combatting the late blight pathogen in the region, without doubt, materially retarded the organism in 1943 and 1944 and resulted in the saving of several million bushels of potatoes.

Ohio

The choice of data that would represent a typical Ohio potato region was difficult because there were a number of rather widely separated areas in which potatoes were grown. Obviously the weather data of all sections

could not be graphically shown. Wooster was chosen because it probably represents one of the largest potato growing areas and also because there are two weather recording stations. The temperature and rainfall data for Stations 1 and 2 were somewhat different. Station 2 recorded the temperature about 2 degrees higher than Station 1 throughout the four-month period under consideration. Station 2, recording the higher temperatures, will be used in this study.

There was only a trace of late blight in 1941. The temperature during June, July, and August was probably too high to permit abundant development of the pathogen since it was approximately 70° F. or above for the entire period.

In 1942 the late blight loss was recorded as 5 per cent although the temperature condition was approximately the same for June, July, and August as in 1941 (1942 temperatures were less than a degree higher than 1941) when only a trace of late blight was observed. It is significant that the rainfall was much lower in 1942 than in the previous year.

In 1943 and 1944 only a trace of late blight was observed in the State. The temperatures for the first three months of the growing season of each year were too high to permit extensive development of the pathogen.

Indiana

Late blight was not recorded in Indiana in 1941. The temperatures for June, July, August, and September for the Fort Wayne region were 69.0° , 74.25° , 73.25° and 68.6° F., respectively, which means that for most of the time the temperature was near or above the maximum for the development of the late blight pathogen. During the same months the rainfall was +0.08 inches above and -1.76, -0.60, and -2.64 inches, respectively, below the normal. The stations at Indianapolis, Terre Haute, and Evansville recorded higher temperatures than the one at Fort Wayne, with the rainfall about the same. Hence the weather for this portion of the State was probably too warm and dry for the late blight organism.

Records for 1942 present a picture different from that for 1941. Late blight was reported in the northern part of the State in late August and was most prevalent in the early part of September. The mean temperatures for June, July, August, and September were 71°, 73.75°, 70.75° and 63.4° F., respectively. The continuous high temperatures for the first three months of the growing season were not favorable for the extensive development of the pathogen, although the rainfall was above normal during this period.

In 1943 late blight also occurred, but the injury to the crop was slight. The disease was most prevalent in September, with an estimated loss of 0.2 per cent (Plant Disease Reporter Supplement 147, p. 177, 1944). The mean temperatures for June, July, and August were 73.0° , 72.75° and 72.0° F. The September temperature dropped to 59.5° . The rainfall in June, July, and August was -1.01, +0.97, and +0.57 inches, respectively.

No late blight was found in 1944. The temperature during the first three

growing months was continuously above 70° F. The rainfall was below normal during June and July, slightly above in August, and -2.1 inches in September. The high temperature and generally low rainfall prevented the development of the late blight organism.

Illinois

Late blight was not observed in Illinois in 1941. The temperatures at Freeport for June, July, August, and September were 69.6°, 74.0°, 72.6° and 66.4° F., respectively. The temperatures for the first three months, especially July and August, were sufficiently high to inhibit the growth of the pathogen. During the same months the rainfall was +0.08 inches above normal for June but below normal for the balance of the season. The high temperature and low rainfall undoubtedly prevented the late blight organism from developing.

The first reported occurrence of late blight in 1942 was in August. The estimated loss was reported as greater than in 1943 (the loss in 1943 was reported as 2.9 per cent). It is reported that late blight was most prevalent during the first half of September. The temperature in June, August, and September was below 70° F., while in July it was 72.6°. The rainfall in July and August was above normal and in June and September, below. The favorable temperature in August and September, coupled with deficient rainfall for the same period, permitted a limited development of the pathogen.

In 1943 the disease was first reported in August and was most prevalent in the first half of September. The temperature was high (above 70° F.) during the first three months and very low in September (58.3°, which was 5.0° below normal). The rainfall fluctuated from above to below normal in alternating months, beginning with a +2.53 inches above normal in June. The temperature range for the season was unfavorable for the development of the pathogen, except in September.

In 1944, there was only a trace (estimated 0.8 per cent) of late blight, which was first reported in late August. The mean temperature for the first three months was approximately 71° F., dropping to 64.6° in September. The rainfall was high in July (+1.08 inches) and below normal in June, August, and September. The weather conditions were most favorable for the development of the late blight organism at Freeport, Illinois, in 1944.

Michigan

Late blight occurred in Michigan in each of the years 1941 through 1944. East Lansing weather data for 1941 (Fig. 1) show the temperature for June, August, and September to be below 70° F. Rainfall for the same three months was above normal. Temperature in Northern Michigan (Onaway) was slightly lower than at East Lansing with approximately the same amount of rainfall. July was warm and dry in both regions. In Western Michigan (Ironwood) the temperature was approximately that of Onaway, but rain-

fall in July was heavier. The first late blight found in 1941 was in Emmet County, about 50 miles west of Onaway.

In 1942 the South-central Michigan weather was somewhat more favorable for late blight than 1941. The temperature during June and July was 67.0° and 70.2° F., respectively, with rainfall above normal for July. Temperature at Onaway was 3 to 4 degrees lower but there was less rainfall. Western Michigan temperature was about the same as that of the northern part of the State, but there was much more rainfall. For example, Ironwood received 10.12 inches in July; Iron Mountain 5.92 inches, compared to 4.33 inches at East Lansing. Late blight was first reported in Northern Michigan (point not specified) August 12. The total loss for the State was estimated as one per cent.

In 1943 East Lansing temperatures were 70° F. or below for the summer, except for a July mean of 70.4°, with June and July wet. Northern Michigan temperatures were 3 to 4 degrees lower on the average, but with lower rainfall for June and July. Somewhat more rain fell in Northern Michigan during August and September than in the East Lansing area. Western Michigan was a little warmer than Northern Michigan, with more rainfall during June, July, and August. The first report of late blight was July 9 in Western Michigan in Menominee County. This early occurrence would be expected, as weather for June and July was more favorable for late blight in Western Michigan than near East Lansing. Loss for the season was estimated at a trace.

In 1944 the weather for the State was somewhat more uniform than in the two previous seasons. Late blight was first reported in Houghton County, Western Michigan, on August 25. Dr. Muncie reported as follows: "...late blight in August and with continued precipitation beginning early in the month with high day temperatures and cool night temperatures late blight became established and caused considerable damage before the crop was harvested in late October." Late blight appeared in Presque Isle County in September and October. The loss to the Michigan crop was less than one per cent.

It may well be that the vigorous control program carried on every year in Michigan materially retards the development of the pathogen, especially when the temperature in June and July is below the maximum for the development of the parasite. The mean temperature for July was always slightly above 70° F. Michigan growers operate 3,500 to 4,000 sprayers and dusters.

Wisconsin

Late blight was destructive in Wisconsin from 1941 to 1944 inclusive. The loss, estimated at 4 per cent, in 1941 was less than for any of the three following years. The disease was first reported August 23 on the late crop in Waupaca County.

The monthly mean temperatures at Antigo for June, July, August, and September of 1941 were 66.1°, 69.70°, 66°, and 60.8° F., respectively. The

rainfall in June and July was low, -2.74 inches and -2.13 inches below normal, while in August and September the rainfall was +8.77 and 2.97 inches above normal. The unusually low rainfall in June and July may well have retarded the development of the pathogen.

The late blight losses in 1942 were high, estimated at 20 per cent in the field and 5 per cent in storage. This loss was over five times that in 1941. The disease was reported in the State about two months earlier in 1942 than in 1941, which meant the pathogen probably had a much longer period in which to develop. It was most prevalent and destructive in late August. The temperature during the growing season was prevailingly lower than in 1941 for June to September, being 63.0° , 66.6° , 65.7° , and 56.3° F., respectively, and the rainfall higher during the early part of the season, being only -0.52 and -0.25 below normal for June and July. It should be emphasized that the mean temperature throughout the season was very favorable for the development of the pathogen.

There was only a 5 per cent loss in the field and about a 6 per cent loss in storage in 1943. The field losses were much less than in 1942, but the storage rot losses were about equal. The disease was first reported on June 6 in Langlade County on the late potato crop. The disease was most prevalent in late August and early September. The temperatures for June, July, and August were $+2.8^{\circ}$, $+1.3^{\circ}$, and $+1.9^{\circ}$ above normal. On the other hand, September was cool, with -4.5° below normal. The rainfall was above normal during the first three months and below in September. The temperature conditions in 1943 were much like those of 1941, being less favorable for the pathogen during the first three months of the growing season than during the same period of 1942. The late blight losses were estimated at 4 and 5 per cent for 1941 and 1943, respectively.

In 1944 the late blight estimated loss was only 2 per cent in the field, but much higher in storage, estimated at 10 per cent. The disease was first reported June 11 in Langlade County, but it became prevalent only in September at about harvesting time. The temperature for the first three months was somewhat higher than 1942 and lower than 1943. It was significant that the August temperature was higher than for any of the three previous years. The rainfall was low in July (-1.4 inches), high in August (+3.5 inches), and low in September (-1.26 inches). The temperature curves for 1942 and 1944 parallel one another with only about a degree difference, conditions that certainly were favorable for the development of the pathogen throughout the season. It is believed that the vigorous control program in effect in 1944 may well have saved thousands of bushels of potatoes.

Minnesota

The potato growing areas of Minnesota are rather widely separated and for this reason no one station's weather data can be used to represent the State as a whole. In this report the temperature and rainfall recorded for Albert Lea were selected since the Red River Valley area could be considered with the North Dakota conditions.

In 1941 the temperature during June was favorable for the development of late blight pathogen around Albert Lea. In the Red River Valley the temperature and rainfall were favorable during June. The first late blight was reported at Brooklyn Center on July 18, but no estimated loss for the State is available.

In 1942 the weather at Albert Lea was even more favorable for late blight. In the Red River Valley there was generally heavy rain, with the mean monthly temperature below 70° F. during June, August, and September and only 0.7° above in July. The first late blight was reported at Hollandale, near Albert Lea. Late blight apparently was general in the State because the estimated loss for the State was 30 per cent.

The temperature was in general less favorable for late blight in 1943 than in 1942. There was less rain in the vicinity of Moorhead and Crookston in the Red River Valley than in the Hollandale area for the same periods, but there was enough rain to provide favorable conditions for the pathogen and some late blight occurred, although the date of first appearance is not available. Loss for the State during 1943 was estimated at 3 per cent.

In 1944 the weather at Hollandale was favorable for late blight for much of the season. The temperature was low during June, July, and August with ample rainfall. At Crookston the temperature was below 70° F. for much of the time but the rainfall was also low. The late blight pathogen was first reported in cull piles at Hollandale but the first field report was at East Grand Forks, July 9, about 200 miles north. The estimated crop loss for the season was between 10 and 15 per cent.

It should be pointed out that in 1941 and 1943 the temperatures were higher during June, July, and August than during the same months of 1942 and 1944 (monthly mean temperatures at Albert Lea). The estimated late blight loss was much less in 1941 and 1943 than in 1942 and 1944.

North Dakota

The temperature and rainfall records at Grand Forks for the four years for North Dakota are in figure 1. The loss in 1941 was recorded as only a trace. The temperature was unfavorable in July but favorable in August and September.

In 1942 the loss was serious, amounting to 25 per cent of the crop. The first report of blight occurrence was on August 5 in Grand Forks County and later it was reported in Walch, Sargent, and Richland counties in the Red River Valley. The temperature was favorable throughout the growing season. The only month when the temperature was at all unfavorable was July when it was above the maximum. The rainfall was below normal in June, July, and September, but above normal in August.

The temperature was generally lower in 1943 than in either 1941 or 1942. The rainfall was very low in August and September, but it was above normal in July. Late blight was first observed on August 10 and was estimated to have caused a 5 per cent loss. It is believed that the very dry

condition in the last half of the growing season adversely influenced the development of the late blight organism.

The weather conditions in 1944 were very different from those in 1943. Instead of being dry during the last half of the season it was very wet. The rainfall in August was 12.16 inches. The temperature was favorable throughout the growing season and late blight was prevalent beginning August 2 in Walch, August 8 in Thraill, August 11 in Cass, and August 23 in Pembina counties. The estimated loss was one per cent on the foliage and 5 per cent in storage. Dr. Brentzel advised by letter that, because of severe hopper damage, most of the vines in these areas were dead and dried by the time the heavy rains fell in August, thus the lack of abundant susceptible foliage prevented the occurrence of a late blight epiphytotic.

South Dakota

Late blight occurred in South Dakota in 1942 and 1943, but not in 1941 and 1944. The temperature at Watertown in July and August was distinctly unfavorable in 1941. The same can be said of the rainfall for these two months.

In 1942 the temperature was generally below 70° F. and late blight occurred in destructive form in the State. It was estimated that the loss for the State was 30 per cent. The disease was first discovered in Deuel and Brookings counties on July 27.

The weather for the growing season in 1943 was warmer than in 1942. The temperature was unfavorable for the development of the late blight pathogen in July and August, with the August mean only $+0.5^{\circ}$ above the maximum for the pathogen. In spite of this unfavorable temperature period, considerable late blight occurred. The loss was estimated at about 13 per cent.

The 1944 season was much like 1942 from the standpoint of temperature and rainfall. The mean temperature throughout the growing season was below 70° F. in each year. The rainfall in 1944 was low in June and July and only slightly above normal for August and September. The late blight loss was, according to estimates, only a trace for this season. The difference in the development of the late blight pathogen in 1942 and 1944 may have been due to the dry June and July in 1944 which prevented the initial development of the pathogen in the potato fields.

Lowa

Late blight has appeared in Iowa in each of the last four years. In 1941 late blight was not observed until in September, when it was collected on tubers in two different counties. Data in hand for 1941 indicate that late blight was neither prevalent nor destructive. The temperature for 1941 during July, August, and the early part of September was above that permitting rapid development of the pathogen. The last half of September, on the other hand, was below 70° F. and favorable for the development of

the pathogen. This unfavorable temperature condition for July and August probably explains why only a small amount of late blight was present in 1941. The rainfall in these two months was low (July +1.1 inches and August -2.2 inches) while in September (+3.4 inches) it was high.

In 1942 the late blight picture and the temperature and rainfall conditions were markedly different from those in 1941. Late blight was prevalent and destructive. In fact, it was estimated that 33 per cent of the potato crop was destroyed. The disease was first found on July 9. The temperature was below 70° F. in June, August, and September, and in July it was only slightly above. The rainfall was rather low throughout the whole period. There was, however, enough rainfall so that the crop never suffered from lack of moisture. This is apparent in that, in spite of the heavy late blight loss, Iowa produced 120 bushels per acre.

The estimated crop loss in 1943 was 16 per cent, about half of that in 1942. The temperature during June, July, and August was higher than in 1942 and July was 3.4 degrees above the maximum for the development of the pathogen. September was cool with little rainfall. Late blight was first found on July 23, about two weeks later than in 1942, and the disease was most prevalent on the vines in late August.

There was less late blight in 1944 than in either 1942 or 1943. The loss in 1944 was estimated as only one per cent. The temperature during June, July, and August was just above the maximum for the development of the pathogen. The temperature conditions in 1944 were more like those in 1941 than either 1942 or 1943. The rainfall was generally high throughout all four months.

The late blight disease during 1941 to 1944 inclusive was most destructive in 1942 and 1943 and least in 1941 and 1944. When the temperature was generally above 70° F. the pathogen was much less destructive than when it was below. The rainfall was lowest in 1942 and highest in 1944. There seemed to be little relation between rainfall and the prevalence and destructiveness of the late blight pathogen.

Nebraska

Late blight was not observed in Nebraska in 1941, although the temperature and rainfall conditions were favorable in June for the development of the pathogen on the early crop. An early crop, grown in the vicinity of Grand Island, is normally harvested in July.

In 1942 late blight was observed at Wood River, near Grand Island, in Hall County, on June 21 on the early crop. The mean temperature for June was 70.4° F. and the rainfall was 7.7 inches. This condition of below normal temperature (-1.6°) and above normal rainfall (+3.65 inches) was favorable for the limited development of the pathogen on the early crop.

The mean temperature in June of 1943 was 0.6° higher than 1942 and the amount of late blight was 5 per cent, some higher than in the previous year. The disease was most prevalent about the middle of July, at harvest time.

The weather condition in June, 1944, was much like that in 1942. The temperature was 71.9° F. and the rainfall 5.44 inches. The late blight disease was very limited, a trace having been observed on August 5 in only one field at Cozad in Dawson County, about 75 miles west of Grand Island.

In Nebraska the late blight situation was very different from that in most other States in the region, because only the early potato crop on mineral soil was diseased. Therefore, it is only the weather condition in the earlier part of the growing season, May and June, that influences the development of the pathogen and the regional forecasting service would not operate early enough to serve that area effectively.

Manitoba

In the absence of temperature data for the Province of Manitoba, no attempt will be made to correlate the temperature and late blight prevalence. The following extract from a report by Dr. J. E. Machacek is an excellent summary of the prevalence and destructiveness of late blight in Manitoba during the four years under consideration:

"In 1941, late blight appeared in Manitoba for the first time since 1928. Rainfall was extremely heavy from August 15 to September 30 with but very little sunshine and many potato fields were covered almost continuously with water. The greatest losses occurred north of Winnipeg but the disease apparently did not spread very far from the vicinity of this city. Early varieties, such as Warba, escaped tuber infection, but in later varieties, such as Irish Cobbler, as much as 7.5 per cent of the tubers in a field were infected.

"In 1942, late blight was again prevalent in the Red River Valley around Winnipeg and in some fields it was severe. The extent of tuber-rotting was difficult to determine because early, severe frost damaged a considerable percentage of undug tubers in September and the occurrence of secondary rots obscured decay from the late blight fungus. In general, the weather during late summer was similar to that in 1941.

"Following a moist, cool July, in 1943, late blight appeared in the Winnipeg area during the early part of August, and spread rapidly for a few days. Its progress was checked toward the end of August and in September by a period of dry weather. Severe tuber-rotting occurred, however, in some areas north of Winnipeg, and some damage occurred in western and north-western Manitoba in fields 150 to 200 miles from Winnipeg.

"In 1944, the weather was very moist and moderately warm during June, July, and August. Late blight was first found in the Winnipeg area in a Victory Garden on July 15 and within a few days it could be found with ease in most of southern Manitoba. By September, the disease had done so much damage that a great many fields were left unharvested. During autumn and winter, rotting tubers were received from practically all agricultural portions of the Province, and also occurred frequently in tubers sold in Winnipeg by retail grocers. The severity of the late blight epidemic in 1944 appeared to be the greatest in the history of the Province.

"The rapid spread of the disease in Manitoba from 1941 to 1944 can be attributed to three principal factors: (1) the unusually moist weather, (2) the unfamiliarity of Manitoba potato growers with the disease during 1941 and 1942, and (3) the current unavailability of spraying materials and equipment."

FORECASTING LATE BLIGHT

Reports from the Sub-committee of the War Service Committee of the Upper Mississippi Valley Plant Pathologists were sent to the chairman at weekly intervals and the data compiled and sent out to pathologists and others interested in potato growing.

In some of the States, seed infected with *Phytophthora* was actually planted so as to afford information on the time of occurrence of the disease. In most cases, however, the development of the pathogen was watched in field plantings and on cull piles near warehouses and on dump piles on the outskirts of towns and villages. The following are a few of the forecasts that were made for 1943. The first forecast to the committee for distribution and use was on June 17, with eight States reporting: North Dakota, Michigan, Wisconsin, Minnesota, Nebraska, South Dakota, Colorado, and Iowa. The forecast was as follows:

"From the standpoint of moisture and temperature, the conditions seem generally to be favorable for the development of the early stages of an outbreak of late blight. In two States the organism has been found fruiting in late gardens and dump heaps. Barring a very sharp and sudden rise in temperature and drop in rainfall for Iowa, late blight will appear in our fields in Early July."

Late blight was found on July 23. The area of vines diseased in the two fields where the disease was first found already comprised several acres, so it is safe to assume that the initial stages of the spread of the pathogen existed from two to three weeks earlier. The forecast on June 17 came true.

The following week, June 26, this forecast was formulated from the data from the same eight States that reported on June 17:

"The late blight organism is developing true to form, and has made a beginning in several places. Information in hand justifies the prediction that the early stages of foci of infection exist. Only a sharp rise in the temperature can stop the organism's increase and spread. Fortunately, the temperature during the past two weeks has been generally upward. Watch the temperature, it is much more damaging to the organism than rainfall."

The next announcement was made on July 19, based on the data supplied by Iowa, South Dakota, Minnesota, Wisconsin, Nebraska, and Michigan. The forecast was as follows:

"The over-all picture is still dark, and conditions are favorable for an epiphytotic over most of the Upper Mississippi Valley. The only bright spots that developed in the first half of July were the high temperatures

in South Dakota and Minnesota. In spite of all this it should be emphasized that a couple of weeks of favorable weather now can induce a widespread epiphytotic. The vines are beginning to close the rows, affording shade and slow evaporation, conditions that favor *Phytophthora*. The only safe thing to do is to keep the sprayers going. If the vines need protection from the tractor wheels, buy or devise vine lifters and keep going."

On July 27 came this warning: "The situation will be serious if a few cool days come. Present weather conditions are hot and dry. With late blight present in fields in North Central Iowa and the weather such as it is in that area, potato districts further north, including the States of Minnesota, Wisconsin, and the Dakotas, are liable to late blight losses unless the temperature rises very sharply and remains well above normal for the balance of the growing season."

North Dakota, South Dakota, Minnesota, and Wisconsin experienced the expected losses. The late blight disease even occurred in the Canadian provinces, although not in destructive form, except in Manitoba.

The last forecast for the season was disseminated on August 4 and reads: "If we can avoid late blight losses we will harvest the biggest crop produced during the last two decades. The early potato crop will be marketed with little loss from late blight, but this will not be true of the late crop. Our early crop always escapes. It is now clear that late blight will do considerable damage to the foliage as the season advances and there will be considerable tuber rot unless the weather turns hot and dry right away. The potato crop in most areas was planted later than usual, which means that it will go into the fall in a vigorous growing condition with lots of foliage, affording an opportunity for a large spore load to sift down into the soil. No amount of spraying can stop this."

The data in table 1 show the following losses in percentage of each State's crop: South Dakota 13, North Dakota 5, Nebraska 5, Iowa 16, Minnesota 3, Wisconsin 11, Illinois 2.9. The forecast of August 4 came true. The basis of the forecast was the favorable weather for the development of the pathogen in June and July.

In 1944 the first forecast was issued on June 5 and read as follows: "Every indication at the present tells us to keep a 'weather eye' on the late blight situation." The prediction of June 5 was strongly supported in the communication of June 26: "Weather conditions have again been favorable for the development of *Phytophthora infestans*. The temperature was slightly above normal except in North Dakota. The rainfall, on the other hand, was low except in Illinois, Wisconsin, Minnesota, and South Dakota."

By July 17 the late blight picture was well outlined, as manifested by this statement: "By this time it is perfectly clear to all of us that late blight, if conditions remain favorable, will cause heavy losses in potatoes that are not sprayed. Fortunately through the splendid work that has been done much spraying has been and is being done. I predict little damage generally in the intensive potato growing regions."

On July 24 came another reminder to the effect that "we must not relax our vigilance for there is still time for the pathogen to cause serious loss."

The situation as of August 14 was as follows: "This season has been an extremely interesting one from the standpoint of late blight. You will recall we started the season with the pathogen general in our seed, which explains the 'popping up' of local epiphytotics in the field from Ohio to Nebraska and from Iowa to Manitoba. Except that the temperature remained close to the upper limit for the development of the late blight organism, we would have experienced a bigger fight in protecting our crop."

The forecast of July 17 was borne out by the following losses: in Wisconsin 12 per cent, Minnesota 10 to 15 per cent, North Dakota, on the vines one per cent and on the tubers 5 per cent. Favorable temperatures in June and July in the States named was the basis for the forecast.

CONCLUSION

Thus, in conclusion, it is possible to summarize by stating that, given the weekly temperature and rainfall of the potato growing areas and assuming that sources of inoculum exist near the potato fields, one can rather accurately predict the prevalence and destructiveness of the late blight pathogen throughout the growing season.

PHYTOPATHOLOGICAL NOTES

A Diplodia Associated with Concealed Damage in Peanuts.—Concealed damage is the name given to an internal deterioration of peanut seed. In the early stages of the disease there is a small dirty white to light yellow spot on the inner face of the cotyledon. As the disease progresses the discoloration becomes more pronounced until the entire embryo is blackened and thoroughly permeated with fungal hyphae, and the cotyledons become rancid. A dense mycelial mat often is between the cotyledons. Only in the last stages of deterioration is the damage evident in unbroken seeds.

The cause of the disease has not been conclusively established. Higgins¹ isolated Rhizoctonia, Penicillium, Rhizopus, and Sclerotium bataticola from seeds with concealed damage and concluded that these soil-inhabitating fungi were responsible for the disease. Results obtained during the fall of 1944 at the Alabama Agricultural Experiment Station do not support this conclusion.

Samples of peanuts were collected from twelve widely separated fields throughout the principal peanut-growing region of Southeast Alabama. All seeds having concealed damage were separated from each sample for isolation of the associated microflora. Disinfecting solutions used were 1-1.000 mercuric chloride, silver nitrate, and 0.5 per cent sodium hypochlorite. Potato-dextrose agar acidified with lactic acid, beef-extract agar, and Czapek's solution agar were used to provide media varying in nutrients and hydrogen ion concentrations. Over 1,200 seeds from the twelve samples have been studied.

Regardless of the method of sterilization or the medium used, the predominant organism obtained was a Diplodia.2 This fungus was obtained from 65 per cent of all seeds plated and constituted approximately 95 per cent of all isolates. Other fungi obtained (5 per cent of the isolates) were Sclerotium bataticola, Fusarium spp., Aspergillus, Penicillium, and Rhizopus; and bacteria also were isolated.

The frequency with which this Diplodia is recovered from damaged seed is strong evidence that it is associated with the disease.—Coyr Wilson, Alabama Agricultural Experiment Station, Auburn, Alabama.

Galls on the Roots of Citron-Watermelon Hybrids.—During the course of studies on the Fusarium wilt disease of watermelons at Leesburg, Florida, it was a routine procedure to pull all plants at the end of the season and observe Fusarium wilt injury on the roots. Included in the 1934 trials were several F₁ hybrids from crosses of watermelons on three strains of African citron obtained from the Iowa Agricultural Experiment Station. When these plants were pulled peculiar galls were observed on the roots of many

¹ Higgins, B. B. "Concealed Damage" of runner peanuts. Georgia Agr. Exp. Sta.

Press Bul. 536. 1944.

2 Tentatively identified as Diplodia theobromae (Pat.) Nowell, by Dr. J. A. Stevenson, United States Department of Agriculture.

of them, particularly on those of three hybrids designated as 427, 430b, and 431. There were no plants of the citron parents in the field, but similar structures were observed later in the same year on the roots of volunteer citron plants on the Experiment Station Farm at Gainesville. Galls were observed on the citron hybrids again in 1935, which was the last year they were planted and none of the galls have been observed since that year. Since these galls were entirely different in appearance from galls induced by nematodes or the crown-gall organism, the observations on them are recorded here.

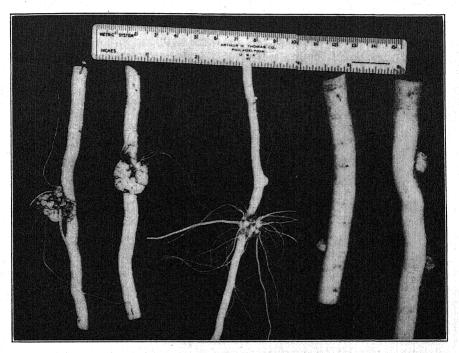


Fig. 1. Roots of citron-watermelon hybrid showing variations in the unusual type of gall.

The galls were most evident on the larger roots but they were also observed on the smaller ones. There was no swelling of the large roots on which the galls occurred, and the growths appeared to be appended to the roots by definite stalks which on the larger galls were comparatively inconspicuous. It is possible that these pedicel-like structures were merely portions of small lateral rootlets. In any event, this characteristic of the galls was a distinct point of difference from the galls induced either by Bacterium tumefaciens Smith and Towns. or by the root-knot nema, Heterodera marioni (Cornu) Goodey.

The galls in the early stages of development were glistening white. This character was less conspicuous later, though still apparent between the fine network of growth cracks that covered the surface of the older growths. In

somewhat larger galls the surface became darkened and slightly scurfy, sloughing off to again expose the fresh sub-surface region of active growth. The largest galls were inclined to be tuberculate and divided, apparently as a result of the irregular enlargement of limited portions of the surface of the growth. This often resulted in a dense cauliflower-like structure that is evident in some of the galls shown in figure 1. Another characteristic of some of the growths was the proliferation of roots around the gall.

Bacteria were numerous in intercellular pockets throughout the superficial layers of the galls. Occasionally nematodes were observed but not consistently or abundantly enough to suggest a causal relationship. A single inoculation series was carried out with cultures of bacteria isolated from the galls, but because galls appeared on the check plants as well as on those that had been inoculated the test was of no value and opportunity for further work has never arisen.—M. N. WALKER, Florida Agricultural Experiment Station, Gainesville, Florida.

REPORT OF THE SECOND ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The second annual meeting of the Potomac Division of the American Phytopathological Society was held February 20 and 21, 1945, at the Plant Industry Station, Beltsville, Maryland. Officers elected for 1945 were T. F. Manns, President; E. E. Clayton, Vice-President; V. F. Tapke, Secretary-Treasurer; and R. J. Haskell, Councilor. Plans are going forward for a one-day field meeting at the Plant Industry Station during the latter part of August.

ABSTRACTS OF PAPERS PRESENTED AT THE SECOND ANNUAL MEETING OF THE POTOMAC DIVISION

Light and Composition of Media as Factors Inducing Sporulation in Strains of Alternaria solani. Beechee, F. S. The fungus, Alternaria solani, which attacks both tomatoes and potatoes, ordinarily sporulates only sparingly, if at all, in artificial cultures. Various workers have induced sporulation by wounding, by scraping, or by irradiation of cultures with ultra-violet light. Considering light as a possible essential factor, closed Petri-plate cultures were exposed to various types of illumination. Sunlight in the greenhouse gave fair sporulation on some media, but was too variable and the heat often too intense. A 300-watt Mazda lamp was without effect. Twenty-four-hour exposure, close to a 3-tube (40 watts each) fluorescent lamp, gave moderate sporulation. Six- to eight-day-old cultures, irradiated under an S-1 sun lamp for 30 minutes or longer at a distance of 30 to 32 inches, sporulated abundantly, especially on bran-extract medium (1 oz. of bran per 1., boiled 10 or 15 min., strained and decanted; 10 cc. per 1. of a 5-per cent solution of ferric chloride; 2 per cent of agar). This extract, alone or combined with juice of green or ripe tomato fruits, gave varying results with different strains of A. solani. Plates kept in darkness showed no sporulation, except in rare instances. Alternaria tomato gave little if any response to irradiation, but eventually sporulated slowly in the laboratory in light or darkness. (Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agriculture.)

Chemical Soil Treatments for Disease and Weed Control in Tobacco Plant Beds. Clayton, E. E., J. G. Gaines, T. E. Smith, and T. W. Graham. Treatments with uramon (urea), 1 to $1\frac{1}{2}$ pounds per square yard, were effective against weeds, black root rot, and root knot. Cyanamid (calcium cyanamide) was effective against weeds but not against black root rot or root knot. Chemicals were applied September-November, and tobacco seed was sown the following January-March. Among other materials tested, D-D mixture gave excellent root knot control but did not destroy weeds or black root rot. The best chemical treatment in 1944 was 1 pound of uramon and ½ pound of cyanamid per square yard. This gave 95 per cent weed control, excellent root knot control, and an average of 21 transplants per square foot. The corresponding yields of plants from uramon and cyanamid used alone were 13 and 15. The better chemical treatments have been superior to steam sterilization with respect to disease control and yield of plants, and inferior with respect to weed control. Best results with chemical treatments have been obtained on light, sandy soils.

Pre-storage Treatments for Seed Sweetpotatoes. Cox, Carroll E., and W. F. Jeffers. Maryland Golden sweetpotatoes were treated before curing and storage for the purpose of reducing losses from decay and preventing spread of diseases during storage. Immediately after digging, potatoes were washed in water and dipped one minute in 4-ozper-gallon solutions of borax, Spergon, and calcium propionate (Mycoban); the same materials in five per cent colloidal silica suspension; and the silica suspension alone. Both washed and unwashed potatoes were included as checks. The potatoes were cured for 12 days at 75-78° F. and a relative humidity of 95-99 per cent and stored at 55-60° F. and a relative humidity of 80-85 per cent. Loss in weight and percentage of rot were determined soon after curing and again after 3 months' storage. Sweetpotatoes treated with borax, borax and silica suspension, and calcium propionate lost significantly more

weight and contained a higher percentage of rotted potatoes than those treated with Spergon, Spergon and silica suspension, silica suspension alone, or either of the checks. None of the treatments significantly controlled storage rots. When bedded Spergontreated potatoes produced sprouts several days earlier than other treatments.

Thielavia terricola from Cotton Fabric and from Soils. DIEHL, WILLIAM W. Thielavia terricola, hitherto with no known conidial stage, is shown to bear amerosporous conidia on germ tubes shortly after germination of the ascospores, but no conidia have been detected in older cultures. The history of the fungus, as shown by specimens and by cultures isolated from soils, from plant roots, and from deteriorated cotton fabrics, reveals a wide distribution in nature. These records, together with the fact that in pure culture Thielavia terricola grows vigorously and fruits abundantly when obtaining its sole carbohydrate nutrition from cellulose, indicate that it is a normal soil organism, capable under favorable conditions of growing upon cotton fabric and some other cellulosic materials. It is probable that this species and the classic Thielavia basicola are identical, but proof is lacking until Zopf's authentic specimens are available for comparison.

Prevention of Decay in Substructures of Houses on Wet Sites. Diller, Jesse D. In providing living accommodations for thousands of war workers and their families, many war-housing projects throughout the country were built on sites that are believed to be too wet. In most cases contact of wood with soil has been avoided. However, particularly where outside temperatures go low enough to promote condensation on sills, there is often need for ventilation in houses that have no heating plant under the floor, in order to keep the wood below a dangerous moisture content. A study was made of 21 houses without basements, in 11 locations in the vicinity of Washington, D. C. Substructure wood moisture, relative humidity, and temperature were determined at approximately 3-month intervals for periods of 2 to 3 years. The results indicate that ½ sq. ft. ventilator opening for each 25 linear feet of foundation wall, plus ½ sq. ft. for each 100 sq. ft. of building area is sufficient. Where it is impracticable to provide adequate ventilation and condensation occurs, tests now in the third winter show that it can be prevented and the wood moisture contents kept below the danger point without ventilation, by covering the soil with asphalt roll roofing. (Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.)

Artificial Transmission of the Virus of Big Vein of Lettuce. Doolittle, S. P., and Ross C. Thompson. All of the writers' earlier attempts to transmit the big-vein virus by artificial inoculations were made with expressed juices of diseased leaves, and no infection occurred. Recent experiments, however, have shown that infection is readily secured when freshly extracted juices of roots of big-vein plants are rubbed on the leaves or pricked into the leaves or stem. Fifty-six of 76 lettuce plants inoculated by these methods have been infected. All plants were grown in steam-sterilized pots and soil. incubation period varied from 36 to 60 days with nearly all affected plants showing symptoms at about 45 days. These periods are about the same as are required for development of symptoms in healthy plants transplanted to infested soil. Drying the roots for 7 days seemed to inactivate the virus. Parallel inoculations, made with juices of mottled leaves of the plants whose roots were used as inoculum, produced no infection. When needle punctures were made through healthy and diseased leaves held in close contact, 1 out of 8 plants showed symptoms within 18 days. This period was so short as to suggest prior contamination of the soil. The experiment is being repeated. All the 80 control plants in the experiments remained healthy. (Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.)

Organization and Germination of Oospores in Several Species of Pythium. DRECHS-LER, CHARLES. Although in their early "lumpy" stage oospores of Pythium oligandrum may show only one refringent body, at maturity they commonly reveal 5 to 15 refringent bodies in the finely granular layer surrounding the single reserve globule. If sparingly irrigated after aging 6 months, they often germinate by extending an evacuation tube, 10 to 50 µ long, through which the contents migrate into a vesicle to be fashioned into zoospores. The oospore of P. periplocum often produces an evacuation tube 50 to 200 µ long, which conducts the protoplasm into a terminal vesicle. In P. salpingophorum, P. vexans, and P. anandrum, the germinating oospore may deliver its contents into a vesicle, or may produce a sporangium; germ sporangia in the latter 2 species are often citriform and distally papillate. After aging 6 months the large globose resting spore of P. undulatum Petersen sensu Dissmann, which contains many reserve globules and plural refringent bodies, germinates promptly on shallow irrigation by extending 1 to 3 germ hyphae whereon are borne collectively 1 to 4 ellipsoidal, often distally papillate, and

occasionally proliferous sporangia. From its internal organization, its two-layered wall, and manner of germination, it may possibly represent a parthenospore homologous to the oospore of *P. ostracodes*. (Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.)

Helminthosporium turcicum Leaf Blight of Coru. Elliott, Charlotte, and Merle T. Jenkins. As a result of the epidemic of Helminthosporium turcicum leaf blight in 1942, field inoculation experiments were conducted at Beltsville, Maryland, in 1944 to determine the relative susceptibility of inbred lines and crosses of dent corn to this disease. About 6 acres of corn were inoculated twice weekly, beginning the last week in June and ending as plants came into tassel. Spore suspensions from a mixture of 5 isolations of the fungus from corn at Beltsville were sprayed into the central whorl of leaves. Rapid spread of the disease began the last week in July and continued throughout the season. Leaf-blight ratings on 200 inbred lines, 175 single crosses, and 162 double crosses indicate that most lines and crosses are susceptible. NC34, CI.23, K175, Ky114, Mo21A, T49B, T105B, and K155 in one group of 100 and CI.15, CI.16 and T×206 in a second group of 100 inbred lines were more resistant than any others tested, showing only traces to light infection. Other lines showed moderate to very heavy infection. In general, the resistance of resistant lines was apparent in their hybrids. Some of the more resistant lines also were resistant in the natural epidemic of 1942.

A Comparison of the Agar Plate and Test-tube-dilution Methods for the Preliminary Evaluation of Fungicides. GOTTLIEB, DAVID. A comparison of sensitivity of agar plate and test-tube-dilution procedures for preliminary assay of fungicides is important. Present investigations reveal that each test gives reproducible values for the toxicity of the chemicals, sufficiently precise for detecting promising materials. However, neither test alone will indicate all materials with good fungicidal activity. For these comparisons the usual agar-plate method has been so altered that the spores are applied to the agar in drops of water from the same collection and at the same spore concentration used in the test-tube-dilution method. Eighty-five different organic compounds were tested against two fungi, Macrosporium sarcinaeforme and Sclerotinia fructicola. in dosage series at 0, 16, 32, 63, 125, 250, and 500 ppm. and the L.D. 50 values determined. With the former organism 49.5 per cent of the compounds had the same toxic values by both The agar-plate method was more sensitive than the test-tube-dilution method for 35.4 per cent and the test-tube-dilution method more sensitive for 15.3 per cent of the compounds. Similar results were obtained with Sclerotinia fructicola. When the L.D. 100 was used for comparison with the agar-plate method, there were still many compounds that gave different toxic values depending on the procedure used. For this reason it may be important to utilize more than one procedure in the preliminary evaluation of chemicals as fungicides.

New Organic Fungicides in the Control of Tomato and Potato Diseases. Heuberger. J. W., AND T. F. MANNS. In 1943 it was found that Spergon, Fermate, No. 604, and Dithane did not control early blight on potatoes; adding zinc sulphate-lime $(1-\frac{1}{2}-100)$ to Dithane increased its protective value over that of Bordeaux and yields were significantly increased by 37 bu. an acre. Research with Dithane on potatoes in 1944 showed the following: (1) addition of lime alone to Dithane did not increase control of early blight or leaf hopper burn; (2) zinc sulphate alone significantly increased control of early blight and hopper burn; (3) zinc sulphate and lime together were not so effective as zinc sulphate alone. In 1944, calcium, sodium, iron, zinc, copper, and lead dimethyl dithiocarbamates (2-100) were evaluated on potato and tomato plants. Zinc dimethyl dithiocarbamate was significantly the best for control of early blight and hopper burn of the potato and of early blight and anthracnose on the tomato; the calcium sait showed considerable promise. Adding zinc sulphate-lime resulted, in general, in significant increases in protective value for the calcium, sodium, and iron salts but not for the zinc, copper, and lead salts. Zinc dimethyl dithiocarbamate was the best of any organic or copper fungicide used for control of hopper burn on potato and of anthracnose on tomato. It was equal to Bordeaux for early blight control on tomatoes and potatoes.

New Developments in Spraying and Dusting Equipment. Hopperstead, S. L. A brief review of the development and performance of the Speed Sprayer, Vertical Boom Attachments, Cornell Sprayer-Duster, California Sprayer-Duster, and the use of Aerosols was presented. The advantages or disadvantages of the equipments as compared to standard commonly used machines was discussed as well as the recent findings regarding present control of pests, the objectives sought and the future possibilities for improvement. The possibilities of adapting the Aerosol principle for control of field and storage

troubles of commercial crops were advanced and experimentation along these lines encouraged.

Periconia Blight of the Hevea Rubber Tree. IMLE, ERNEST P., AND JOHN A. STEVENSON. A new disease, occasionally causing conspicuous damage to the Hevea rubber tree, has been reported from Mexico (unpublished) by W. J. Martin and from Costa Rica by the writers. An unnamed species of Periconia, having exceptionally large spores, was identified on such diseased leaf specimens of Hevea spruceana and H. brasiliensis collected at Turrialba, Costa Rica. Symptoms consist of leaf spots and leaf, petiole, and twig blight on H. spruceana but seem to be confined to leaf spots and blight on H. brasiliensis. Leaf spots are circular or elongated along leaf veins and from 2 to 10 mm. or more in diameter. On young leaves the spots often coalesce involving an entire leaflet and causing abscission. Both surfaces of the necrotic spots show the presence of large, dark, septate conidiophores. These, together with their apical cluster of spores, measure 200 to 300 μ tall and are easily visible to the naked eye on close inspection. Infection has been readily obtained on H. spruceana and on spruceana × brasiliensis hybrids by atomizing with a spore suspension and by suspending diseased leaves above succeptible foliage. In one test, spores were being produced on the newly formed lesions 10 days after inoculation.

The Value of Several Fungicides as Sweetpotato Seed and Sprout Treatments. Jeffers, W. F., and C. E. Cox. Maryland Golden sweetpotatoes dipped in solutions of Borax, Spergon, and compound No. 604, before bedding, produced significantly more sprouts at the first pulling than untreated and mercuric chloride-treated. Total sprouts from 3 pullings show all treatments significantly better than check. Addition of Nu-Film, a rosin-residue sticker, delayed production but significant differences were still obtained. Sprouts from untreated sweetpotatoes were dipped in various solutions at the rate of 1 lb. of material per 10 gal. Six weeks after planting, Fermate plots showed significantly better stand than check; both Fermate and Spergon were better than Semesan Bel at odds of 99: 1. Fusarium-wilt infection was reduced significantly by all treatments. Spergon- and Fermate-treated sprouts yielded significantly better than Thiosan and Semesan Bel at the 5 per cent point and better than the Mycoban (calcium propionate) and untreated plots at the 1 per cent point. Results were not so good when the same treatments were used in a paraffin base.

Elsinoë piri Discovered on Apple and Pear in Western Washington and Oregon. JENKINS, ANNA E., M. J. FORSELL, AND L. W. BOYLE. The conidial stage (Sphaceloma) of what is evidently Eslinoë piri, causing a leaf and fruit spotting of apple and pear, was discovered in the United States in 1943. It was found in western Washington by the Special Pest Survey of the U. S. Bureau of Entomology and Plant Quarantine. The following year a survey by the Emergency Plant Disease Prevention Project revealed its presence on both apple and pear in western Oregon. At present, so far as is known, the disease is confined to the moist sections of these States and is not known to occur in commercial apple-growing sections. Trees on which the disease was found are in home plantings, generally not of recent origin and practically without cultural attention. The characteristic fruit and leaf spot was seen on old apple varieties, names now unknown, as well as on Ortley, Grimes Golden, and King, and also on apple seedlings. Late yellow varieties and seedlings with light-colored fruit appear to be particularly susceptible. As many as 100 spots, up to 2 mm. in diameter, have been counted on a single small fruit; characteristically, these were present chiefly on the blush side. An equal number of spots, 0.5 mm. to 1 mm. in diameter, have been noted on a more or less limited area of a single leaf blade. Leaf spots may reach 3 mm. in diam. The assembled data indicate that the apple and pear disease has been present in western Washington and Oregon for an extended period.

Further Studies on Bacterial Leaf Blight and Stalk Rot of Corn. Johnson, A. G., Alice L. Robert, and Lillian Cash. The bacterial leaf blight and stalk rot of corn, previously reported from Alabama and Virginia, has now been reported also from Georgia, Texas, Kansas, and Nebraska. In some Kansas fields it was reported as rather common in 1943 and 1944, although not particularly destructive in either year except in localized spots. In the recent reports, the stalk-rot symptoms have been less common than originally reported from Alabama. The leaf symptoms have been very similar but the severity of the attacks has been lighter than in Alabama. The causal organism is very similar to if not identical with Pseudomonas alboprecipitans, originally isolated from Setaria lutescens. The organism has been shown pathogenic on leaves of dent corn, sweet corn, wheat, barley, rye, and oats; and to some extent on sorghum, Sudan grass, Setaria lutescens, and S. geniculata. The best infections were obtained when greenhouse temperatures were relatively high. Seed of dent corn and sweet corn, inoculated with water suspensions of

the organism, produced seedlings with primary lesions from which the typical organism was isolated. Water suspensions of the organism also caused the typical stalk rot in field and greenhouse.

Observations on the Bacterial Canker of Cowpea. Lefebure, C. L., and Helen S. Sherwin. Burkholder in 1944 described Xanthomonas vignicola as the cause of bacterial canker of cowpeas, while in the same year Hoffmaster suggested that it may be caused by Bacterium vignae. The writers have made isolations from specimens of Blackeye cowpeas sent from Texas, and from Whippoorwill and Chinese Red cowpeas collected in Florida. Both yellow and white bacteria were obtained, but only the yellow organism proved to be pathogenic. When grown on different media, the yellow organism agreed with the description given by Burkholder for X. vignicola. The first symptoms observed after inoculation are extremely variable, depending somewhat on method of inoculation. On primary leaves, small white spots 1-2 mm. in diameter develop. The tissue in these small spots shrivels, drys, and often drops out, producing a shot-hole effect. A halo usually forms around these spots and the invaded tissue becomes water-soaked. The lesions may enlarge to an inch or more in diameter before the leaf wilts. Often the primary and trifoliate leaves wilt without any apparent lesions. On stems, cankers may be the first observable symptoms, and cankers may appear anywhere on stems. Inoculations have shown that varieties differ greatly in relative resistance.

Races of Helminthosporium turcicum. Lefebvre, C. L., and Helen S. Sherwin. Cultures of Helminthosporium turcicum isolated from common Sudan grass, Atlas sorghum, Johnson grass, and corn differed in their ability to infect the host testers employed. Two cultures from common Sudan grass and one from Atlas sorghum were pathogenic on Sudan grass and Gooseneck sorghum, but all failed to infect corn (Hy $\times 540$). Four of the cultures from corn infected common Sudan, while two did not, all six being highly pathogenic on corn (Hy $\times 540$). The culture from Johnson grass caused heavy infection only on its own host. Hence, these ten cultures represent four distinct parasitic races. The fact that many of the isolates from corn are able to produce heavy infection on common Sudan grass may help to explain why Sudan grass is always so heavily infected by H. turcicum when grown in Eastern United States. On the other hand, although only a few cultures from Sudan grass and sorghum were used, these failed to infect corn. This may account for the frequent absence of the fungus on corn growing close to heavily infected Sudan grass.

A Red Leaf Disease of Field and Sweet Corn. Manns, T. F., and C. E. Phillips. In working with leaf blights on many strains of hybrid and open-pollinated corn in 1943 and 1944, we observed a disease, new to Delaware, that we are calling the red leaf disease of corn. It is a systemic disease, characterized in its early stages by brilliant red foliage, turning in its later stages to a brilliant purplish-red, involving the whole plant. There are no necrotic lesions, nor any quick wilting areas. Plants from 2 feet in height to full-size were infected. The ear, though present, never completed kernel formation. In this respect the disease causes total loss. In one plot of 44 strains at Georgetown, 35 sources were infected, that is 79.3 per cent; the amount of infection varied from 1.7 to 11.7 per cent with an average of 4.9 per cent. In 1943, at Newark, 37 per cent of a sweet-corn planting was affected. The cause of the disease is unknown to the writers. Our review of corn diseases shows none similar in symptoms. Cultures of surface-disinfected leaves (1-3000 alcoholic bichloride of mercury) frequently gave an abundant bacterial growth having colonies with a metallic lustre similar to that of Bacterium pruni. We have made no inoculations. Is the disease bacterial, virus, or an inherited breakdown?

Chlorosis in Seedlings of Hevea brasiliensis. Martin, W. J. Less than 0.1 per cent of the Hevea seedlings growing in nurseries in Mexico and Guatemala has been observed to exhibit a partial chlorosis, ranging in severity from a few well-defined spots on a few leaves to almost complete chlorosis of most of the leaves on some affected plants. With very young leaves the condition appears as lighter green spots, mostly with irregular margins. As the leaves mature the affected parts usually lose all chlorophyll and become yellow or white. In severe cases the young stem may appear yellowish green or streaked with yellow. Different leaf flush cycles on the same plant may show different degrees of chlorosis. The chlorosis has been propagated through patch budding; 30 of the 32 buddings, made with buds from affected seedlings placed on normal green seedlings, developed shoots showing the condition. Different degrees of chlorosis appeared among buddings made from the same budwood stick. In 1 of 6 cases, a bud from a normal green seedling, budded on an affected seedling, developed a shoot with a similar chlorosis on a single leaf in the third leaf flush cycle. This suggests that the condition may be transmissible.

Salts as Antidotes to Copper in its Toxicity to Fungi. MARSH, P. B. Spore-germination experiments with Stemphylium sp., Curvularia sp., and Penicillium sp., after the man-

ner of earlier experiments with Sclerotinia fructicola (Phytopath. 35: 54–61, 1945) reveal pronounced antidoting effects of calcium chloride toward the toxicity of copper sulphate. Experiments with Stemphylium indicate that the minimum ratio of calcium to copper necessary for observation of the antidoting response increases with increasing copper concentration. At a given copper concentration KCl is less effective than CaCl₂. Germination of spores of Stemphylium in water suspension on a film of copper hydrogenated resinate may be raised from zero to over 90 per cent by the use of an antidoting salt.

The Use of Easter Lily Bulb Scales for Evaluating Fungicides. McCLELLAN, W. D., AND NEIL W. STUART. The results obtained when Easter lily scales (Lilium longiforum) are treated with various fungicides and growth substances for the control of scale rot caused by Fusarium oxysporum f. lili, suggest their use as a test object for the evaluation of fungicides. The use of Easter lily scales has these advantages: all scales from one clon have the same genetic constitution; clons differ in susceptibility to scale rot, so that both susceptible and resistant clons may be employed; resistant clons provide a measure of the growth-promoting properties of the materials tested; bulbs can be used at any time of the year if properly handled; the organism readily infests the sand on which the scales are placed and the lesions it produces on the scales can be seen with ease; the materials to be tested can be applied as a dust, a suspension, or a solution; the equipment is simple (Phytopath. 34: 966–975, 1944); and the method is rapid, gives reproducible results, requires very little space, and measures small differences accurately because of the low variability between replicates.

Observations on Lima-bean Scab in Puerto Rico. McCubbin, W. A. Lima-bean scab (Elsinoë phaseoli) occurs in Puerto Rico on cultivated and semi-wild Lima beans, and has been frequently encountered in the inspection of pod Lima beans offered for shipment to the mainland since 1930. Observations in Puerto Rico during 1935–39 indicate that, following pod infection, there is a rapid expansion of the scab spot, which at this stage is slightly raised, remains uncolored, and produces a copious but transient crop of superficial conidia; that color appears upon an early and complete transition from the conidial to the ascosporic stage with little, if any, increase in spot size thereafter; that pod infection was obtained with difficulty from ascospore suspensions; that the conidia may be largely responsible for field spread; and that in frozen and unfrozen scabby pods subjected to moist decay the asci and ascospores appeared to become disorganized along with the host tissues, suggesting the possibility that the fungus may not be adapted to carry over in dead plant parts, and thus might have difficulty in bridging the interseason gap in cold regions. (Bureau of Entomology and Plant Quarantine, Agricultural Research Administration.)

Virus of Cucumber Mosaic Withstands Desiccation in Leaf Tissue. McKinney, H. H. When finely cut leaf tissue had become 'dry' in laboratory atmosphere at 18.5° to 23° C, virus activity could not be detected, but when dried at 35° C and stored over CaCl₂ in a disiccator at 23° C, there was slight activity after 58 days. However, when the tissue was dried and stored at 1° to 2° C, over CaCl₂, there was much activity after 153 days. In the laboratory, in humid climates, leaf tissue does not become completely dry, as it does in a desiccator. It appears that slow incomplete drying at laboratory temperatures favors reactions of plant constituents, possibly enzymes, that are deleterious to the virus, and that these reactions can be reduced or possibly eliminated in a very dry atmosphere at suitable temperatures.

A Leafspot of Cowpea and Soybean, Caused by a New Species of Helminthosporium. OLIVE, LINDSAY S., DOUGLAS C. BAIN, AND C. L. LEFEBVRE. A species of Helminthosporium, apparently heretofore undescribed, was found causing severe spotting of cowpea leaves in Louisiana in August, 1944. Since then specimens of cowpeas, as well as soybeans, diseased by the same fungus, have been received from North Carolina, South Carolina, and Florida. The fungus was obtained in culture, and inoculations were carried out on several varieties of cowpea and soybean. All plants became infected, but the cowpeas sustained the greater amount of damage. Two parasitic races have been isolated. Race 1, originally isolated from cowpea leaves, causes a severe spotting of cowpea leaves and a light spotting of soybean leaves; whereas race 2, originally isolated from soybean leaves, produces a light spotting of soybean leaves and few to many small dots of little consequence on cowpea leaves. The fungus is a rather atypical species of Helminthosporium, with long tapering conidia, formed singly or in chains under proper conditions of temperature and moisture, at the tips of long, dark-brown conidiophores. We are applying to the disease the common name of target-spot, with reference to the concentric zonation in each leaf spot on the cowpea, which is the chief host.

Distribution of Races of Tilletia foetida and T. caries in Relation to the Wheat Improvement Program in the United States. Rodenhiser, H. A., and C. S. Holton. Fifteen

races of Tilletia foetida and 16 of T. caries have been identified from 369 bunt collections from 35 States in the United States, 6 States in Mexico, and 2 Provinces in Canada. The most prevalent and widely distributed races of T. foetida are L-1, L-2, L-3, and L-4; and of T. caries T-1, T-2, and T-4. The first 3 and 2 of each group, respectively, are pathogenic only on the old commercially grown varieties. Several other races of both species, pathogenic on the newer varieties bred primarily for smut resistance, are rather widely distributed. Apparently the distribution and prevalence of specific wheat varieties are the most important factors governing the distribution and prevalence of particular races. It seems evident from wheat varietal tests with the above races that the so-called Hope or H-44 factors supply adequate resistance for the spring wheats, while the combined factors from Oro and Martin supply the necessary factors for resistance in the winter wheats. Both Ridit and Oro possess factors governing resistance to all of the races thus far identified in collections from Mexico. (Cooperative investigations between the Division of Cereal Crops and Diseases, U. S. Department of Agriculture and the Washington and Idaho Agricultural Experiment Stations.)

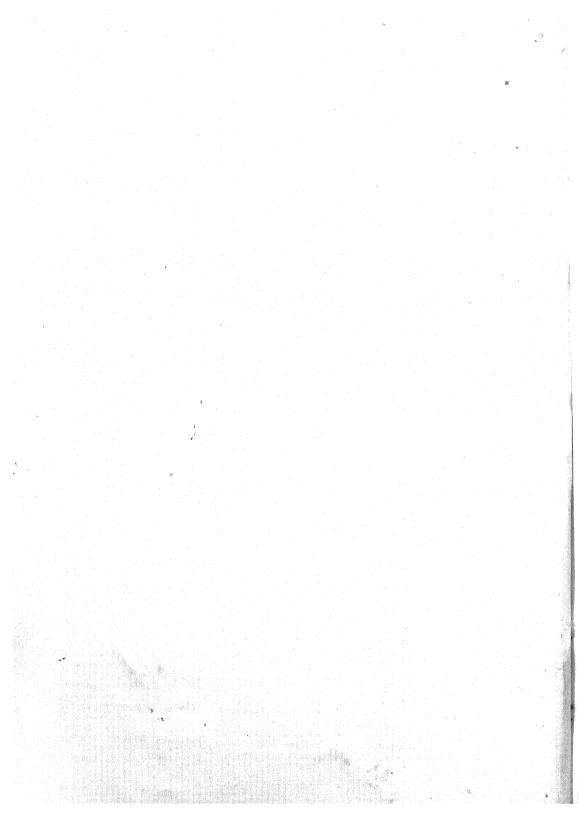
Meadow Nematodes as the Cause of Root Destruction. Steiner, G.

Why Soil Fumigation? STEINER, G.

Phloem Necrosis Research During 1944. SWINGLE, ROGER U., B. S. MEYER, AND CURTIS MAY. Resistance tests have been in progress since 1942 on seedlings and clones of a few American elms (Ulmus americana) suspected of being resistant to phloem necrosis because of their survival in areas where the disease has occurred for many years. Results as yet are not conclusive but they indicate that many of these seedlings and clones are resistant to phloem necrosis. Some natural hybrids between Ulmus fulva and Ulmus pumila seem to be resistant also. Preliminary physiological investigations of inner bark indicate important differences in oxidizing enzyme activity, oxygen consumption, and water relations between diseased and healthy phloem. Electron microscope studies failed to reveal any differences between phloem exudates from diseased and healthy trees. demics of phloem necrosis are now developing across Missouri and in eastern Kansas. The disease area is now known to include the southern halves of Ohio, Indiana, Illinois, and Missouri, eastern Kansas, western West Virginia, Kentucky, western Tennessee, and Throughout this area losses among both wild and planted elms northern Mississippi. were extremely heavy in 1944. (Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.)

Occurrence and Distribution of Physiologic Races of Ustilago hordei in the United States. TAPKE, V. F. In a study of 444 collections of barley covered smut from 33 States, 13 distinct physiologic races of Ustilago hordei were isolated by means of the 8 differential varieties, Excelsior (C.I. 1248), Hannehen (C.I. 531), Himalaya (C.I. 1312), Lion (C.I. 923), Nepal (C.I. 595), Odessa (C.I. 934), Pannier (C.I. 1330), and Trebi (C.I. 936). Race 6 was by far the most common. It occurred in 272 of the 444 collections and was found in 28 of the 33 States. In California and Washington, however, race 5 was very prevalent, occurring in 42 of 45 collections from the former and in 21 of 29 from the latter. In the winter-barley region, races 1 and 6 were common. Races 1, 5, and 6 comprised 86.5 per cent of the total collections. The knowledge of the relative importance of these 3 races and of the occurrence and distribution of all 13 races should appreciably facilitate breeding for resistance against the covered smut of barley, especially since we now have an effective artificial method of inoculating the seed with U. hordei.

Control of Bean Rust by Fungicide Dusting and Spraying. Zaumeyee, W. J., and M. C. Goldsworthy. Sulphurs (wettable and liquid), dithiocarbamates, and chlorinated naphthoquinone proved to be highly efficient in greenhouse experiments in the control of rust infections. Copper compounds were not so effective as the above materials. Liquid lime-sulphur, chlorinated naphthoquinone (604), and disodium ethylene bisdithiocarbamate (Dithane) proved especially effective in eradicating the fungus from infected bean plants (24-hour infection). In field studies, dusting with finely ground (325-mesh) sulphur at the rate of 20-25 pounds of sulphur per acre, before and immediately following the field observation of primary rust infections, controlled the disease; large reductions in yield were observed in unprotected plantings. The field dusting was done during the evening, or the quiet hours of day or night, or in the early morning. Dusting was not done during the hours when dew or precipitation was present on the vines because of the hazard of spreading bacterial blight. (Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.)



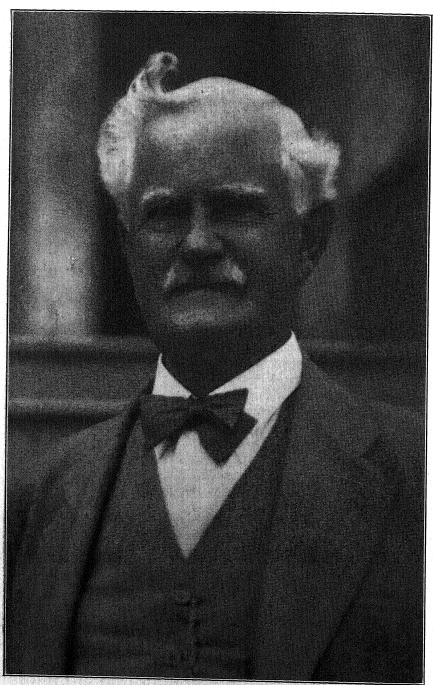
PETER HENRY ROLFS 1865–1944

HOWARD S. FAWCETT

Peter Henry Rolfs, who was among the pioneer plant pathologists of America, and a charter member of the American Phytopathological Society, came to the evening of a life of great usefulness to botanical and agricultural science at Gainesville, Florida, February 23, 1944. He was born on April 17, 1865, at LeClaire, Iowa. A course of study at the Iowa State College at Ames followed the earlier years on his father's Iowa farm. His B.Sc. degree at Iowa State College was obtained in 1889 and his M.S. in 1891. The University of Florida honored him with a Doctor of Science degree in 1920.

Following his studies at Iowa State College, he became Professor of Natural Science at the Florida Agricultural College in Lake City from 1891 to 1899. At Clemson College, South Carolina, he was botanist and bacteriologist from 1899 to 1901. In 1901 he was officially known as Plant Pathologist in accepting a position with the United States Department of Agriculture at the Sub-tropical Plant Introduction Garden, Miami, Florida. He seems to have been the first to successfully bud and graft avocados. He was appointed to the Directorship of the Florida Agricultural Experiment Station at the University of Florida, at Gainesville, in 1906, where he remained until 1921. It was during the first years of his directorship (1906) to 1912) that I learned to know and appreciate his personality and worth and to receive the benefit of his experience, enthusiasm, and his helpful, kindly advice in carrying on research in the then newly differentiated field of plant pathology. I was, I believe, his first graduate student. Added responsibility came later to Professor Rolfs in being appointed Director of the Agricultural Extension Division in 1913 and Dean of the Agricultural College of the University of Florida in 1915.

When a request came to the United States Department of State from Brazil for a man best fitted to found a new agricultural college along practical applicational lines, Dr. Rolfs was chosen. In 1921 he went to Brazil and began the development on a new site in the State of Minas Geraes at Viçosa, the institution now known as Escola Superior de Agricultura. This institution, the building of which he supervised with great pains and attention to details from the ground up, is a real monument to Dr. Rolfs' memory. At the same time, it is one of the most valuable and practical contributions to international good will. The esteem with which he was held after his retirement in Brazil in 1933 is shown by an effort to highly praise one of his successors in saying "this man gives promise of being a second Rolfs." Another indication is that, in 1943, a bronze bust of Dr. Rolfs was placed at the front entrance of the main college building at Viçosa.



PETER HENRY ROLFS 1865-1944

The lasting influence of his work in Brazil is indicated by the fact that hundreds of agriculturists, not only from Minas Geraes, but from other states of Brazil, attend a "Farmers Week" at the college. The attendance at Farmers Week passed the 900 mark in 1944. The regular college attendance in 1943 was 1200. This is, no doubt, having far reaching influence among the solid citizenry of Brazil. No small part of this work by Professor Rolfs in Brazil is to be attributed to the help of his daughter, Clarissa Rolfs, who was constantly with him and became even more proficient in the Portuguese language than he. With her he shared his agricultural interests and published 20 or more papers jointly.

He was made "Consultor de Agricultura" in the State of Minas Geraes from 1928 to 1933, the time of his retirement. In 1892 Dr. Rolfs married Miss Effie Stone who died in 1929, leaving two daughters and four grandchildren. He stayed in Brazil for several years after his retirement with one of his daughters, Miss Clarissa Rolfs.

He was a member of the following scientific societies and organizations: Emeritus Life Member, American Association for the Advancement of Science, 1938; charter member of Botanical Society of America, and of The American Phytopathological Society; Honorary Life Member, Florida State Horticultural Society, 1920; President, 1907–8, Chairman Executive Committee, 1908–21; Member International Association of Botanists; St. Louis Academy of Science; Iowa Academy of Science; Association for Promotion of Horticultural Science; American Pomological Society; Associate Member, American Association of Economic Entomologists; State Chairman, Council of National Defense, 1917–18; and member of Alpha Zeta, Phi Kappa Phi, and Sigma Xi.

That Professor Rolfs was one of the pioneers in mycology and plant pathology is shown by some of his publications. In "A fungus disease of the San Jose scale" in 1897, he opened up the subject of fungi as part of the biological control of insects in Florida which paved the way for later investigations in this field. He gave attention early in Florida to diseases of economic crops: tomatoes 1898, 1907; oranges 1898, 1904, 1906, 1908. He carried his interest in plant pathology to Brazil where, although his main interest was horticulture, he yet found time to give some attention to diseases. He established at the institution in Viçosa a department for teaching and research in plant pathology, headed by a plant pathologist from the United States, A. S. Müller. The breadth of his interests is shown in his papers ranging from "Lichens in Florida" for the St. Louis Academy of Science to "Sub-tropical vegetable growing" in his book published by Macmillan Company and "Domesticating anti-lepric species in Brazil" in the Leprosy Review.

The scope of his interests in plant pathology, as well as other agricultural fields, may be gained by the long list of his publications:

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1938.

STUDIES IN THE FUSARIUM DAMPING-OFF OF CONIFERS. III. RELATION OF TEMPERATURE AND SUNLIGHT TO THE PATHOGENICITY OF FUSARIUM¹

HOWARD TINT2

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Comparatively little attention has been given to the relation of temperature and sunlight to damping-off as predetermining conditions affecting the levels of pathogenicity in the host-pathogen relationship. The close interrelationship in the field between temperature, sunlight, and moisture factors has made it difficult to interpret observations in terms of any one factor. Only when these conditions are controlled and varied independently in the laboratory, can their relations to the disease be interpreted.

GENERAL MATERIALS AND METHODS

The selection of hosts and damping-off fungi was based upon results from previous experiments (22, 23). In the reports on these tests, sources of seeds and of the Fusarium lines were described in detail, as well as standard methods of seed sterilization, selection of monosporous cultures of virulent strains of fungi, and preparation of inoculum and glycerophosphate nutrient solutions for the hosts and fungi. Damping-off losses were considered in two categories, emergence loss and post-emergence damping-off. Where experimental procedure required occasional departures from the standard methods of these early experiments, the alternative treatments are described.

All seedlings were grown in a low-humidity greenhouse, thermostatically adjusted to maintain a daily mean temperature range of 68-75° F., in those seasons of the year when such control was possible.

Determinations of pH were made potentiometrically with quinhydrone and saturated calomel half-cells, and all solutions were adjusted in acidity with 0.1 N HCl and 0.1 N NaOH.

TEMPERATURE

Various writers have considered effects of soil temperature on seed germination and early growth of forest-tree seedlings (1, 6, 14). Excessively high temperatures following high insolation exert lethal effects upon coniferous seedlings (2, 9, 25). Less attention has been given to the effects of moderate temperatures in predisposing seedlings to damping-off, although it has been generally recognized that temperature variations influence the activities of damping-off and other soil organisms (17, 21).

Fusarium species in general are adapted to a wide range of temperature, but usually develop best at relatively high temperatures, with optima in the

¹ A portion of a thesis submitted to the faculty of the Graduate School of the University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

² The greater part of this investigation was carried on during the tenure of a George Leib Harrison Fellowship at the University of Pennsylvania.

range 20-35° C. (12, 16, 26). Wollenweber (27) made the generalization that root-invading Fusaria are warm-soil organisms. Other investigators (11) saw a correlation between high-temperature Fusarium wilts and the vegetative development of the parasites, and attributed the influence of temperature to its direct effect upon the fungus. Gifford (4) and Hartley (10) observed parallelism between high temperatures and damping-off of conifers, and Roth (18) demonstrated that damping-off fungi function actively between 11° and 30° C. His two species of Fusarium caused appreciable damage to Picea excelsa under the protracted influence of temperatures between 24° and 33° C.

The influence of temperature on the development of the disease, and upon the host and pathogen separately, was studied in the greenhouse in temperature chambers described by the writer (24). Air temperatures fluctuated about means at five different levels in a manner resembling diurnal fluctuation in nature. The actual mean temperatures and their standard deviations were as follows: Chamber I, $31.5 \pm 1.3^{\circ}$ C.; II, $24.6 \pm 1.6^{\circ}$ C.; III, $20.9 \pm 1.4^{\circ}$ C.; IV, $16.4 \pm 1.5^{\circ}$ C.; and V, $10.4 \pm 1.6^{\circ}$ C. The standard error of any mean was 0.3° C. These values were independent of the greenhouse temperatures.

The first inoculation tests (Series 1) were upon *Pinus resinosa* in quartz-sand cultures. Two four-inch pots, inoculated with cultures of *Fusarium oxysporum*, and two control pots, treated with sterile rice-mush, were in each chamber. Infested pots were incubated for 5 days at room temperature, after which 50 surface-sterilized seeds of the host were sown in each and covered with sterile sand. The pots were then placed in the apparatus and kept uniformly moist by frequent watering with the standard nutrient solution adjusted to pH 6. Data recorded for inoculated and control pots are in tables 1 and 2.

In table 1 are listed the cumulative percentages of seedlings emerging after various periods, up to 125 days, after placement of pots in the chambers. Since emergence had not been completed by 125 days in Chambers IV and V, it was necessary to determine the number of viable seeds remaining in these pots, in order to distinguish between lack of emergence due to inoculation and that due to physiological effects upon the seeds of the temperature alone. The sand in all the pots was sifted through a screen and all unopened seeds recovered were again surface-sterilized and planted in sterile agar in Petri dishes. The plates were incubated at room temperature for 15 days, and the additional germination was used to correct the percentage emergence of the temperature treatments. The corrected emergence percentages are in table 2, which includes the percentage reduction in emergence due to the inoculation, based upon the corresponding controls as standards, and also the total post-emergence losses.

The optimum temperature range for the emergence of the host was 16–20° C., higher values reducing germination, while lower temperatures slowed down its rate. Reduction in emergence due to the effects of the inoculations

TABLE 1.—Emergence percentages of seedlings of Pinus resinosa grown in control and inoculated quartz-sand cultures at various temperatures. Data are averages of duplicate trials

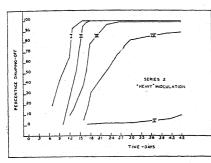
Culture	Tem-	Emergence percentages from 9 to 125 days after planting of seeds									Total emergence
	degree C.	9	12	14	16	18	40	75	100	125	(per cent)
	31.5	6	13	17	21	24				.,,,,,	24
	24.6	5	27	$\overline{32}$	35						35
Control	20.9	ī	39	57	65	73	75	75	76		76
Compros	16.4				2	5	24	44	56	70	70
	10.4						3	7	9	12	12
	31.5										0
	24.6	4	9	11	*****					.,	11
F. oxysporum	20.9	4	13	16	24	27	29	31			31
3.2	16.4				4	6	27	38	47	52	52
	10.4						3	7	12	14	14

was directly correlated with temperature, maximum losses occurring in the warmest chamber and minimum in the coldest. A similar correlation with temperature was evident for post-emergence losses.

Some seedlings failed to survive in the control pots, particularly at the warmer levels. Fusarium oxysporum and other fungi and bacteria were isolated from such dead seedlings. Nutrient-saturated cultures maintained for a long time in closed chambers at high temperatures provided a highly suitable environment for the development of a fungal flora. Whether failures to survive in such controls were due to lethal effects of temperature upon the host, to the saprophytic organisms entering upon death of the host, or to direct pathogenic attack by these organisms upon the weakened host, could not be determined. Infection of controls with F. oxysporum could be directly attributed to contamination as a result of the surface-watering of inoculated and control pots in close proximity. By exercising special care in handling and watering pots while in the chambers, losses in control pots in later tests were reduced to comparatively low levels.

TABLE 2.—Corrected emergence percentages and post-emergence losses in seedlings of Pinus resinosa grown in temperature chambers

Culture	Tem- perature, degrees C.	Corrected percentage emergence	Emergence as percentage reduction of control	Percentage post- emergence loss
	31.5	24		61.1
	24.6	35	alaga ilga mmini alaga ilga	27.9
Control	20.9	76	.,,	11.9
	16.4	77		1.3
	10.4	74	Carlotte Statement	0.0
	31.5	0 .	100.0	
	24.6	11	68.6	100.0
F. oxysporum	20.9	31	59.2	89.6
	16.4	54	29.8	8.7
	10.4	70	5.4	0.0



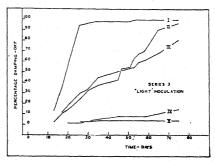


Fig. 1. Effects of temperature on damping-off in seedlings of *Pinus resinosa* in quartz-sand cultures with "heavy" inoculations and with "light" inoculations of *Fusa-rium oxysporum*.

Since seeds germinated at different rates in the various chambers, the emerged seedlings were exposed for different lengths of time to the fungus. Therefore, the degrees of post-emergence attack at the several temperature levels were not wholly comparable. In order to allow uniform exposure of the seedlings with respect to time, two additional experiments were made (Series 2 and 3). Seeds of Pinus resinosa were germinated under sterile conditions and the seedlings transplanted, when their radicles were 1-3 cm. long, to inoculated and control pots which were then placed in the chambers. In Series 2, the standard moderately heavy inoculation procedure was employed. In Series 3, this method was modified as follows: the contents of a flask of rice inoculum were shaken with 50 cc. of distilled water, and the suspension was then used to inoculate two pots of sand. Controls were similarly prepared with a suspension from sterile steamed rice. Thus, inoculation was relatively light in Series 3. Damping-off losses were recorded periodically during 45 days in Series 2 and for 75 days in Series 3. The results are in figure 1.

Upon completion of these tests, the seedlings were removed from the control pots and certain growth responses to the different temperatures were

TABLE 3.—Effects of temperature upon the growth of seedlings of Pinus resinosa in quartz-sand cultures

Series	Tem- perature,	Number of seed-	Averag	e length imeters	Average in millig		Ratio green	Survival in per-	
and duration	degrees C.	lings measured	Roots	Hypo- cotyls	Green	Dry	to dry weight	centage of original stand	
	31.5	19	14.7	40.7	50.5	3.8	14.5	78.0	
a	24.6	30	15.8	40.9	45.8	5.0	9.2	100.0	
Series 2,	20.9	30	19.7	42.6	43.8	5.3	8.3	98.0	
45 days	16.4	30	17.0	39.4	40.7	5.4	7.5	100.0	
	10.4	30	16.3	36.9	38.1	4.9	7.8	100.0	
	31.5	12	18.0	53.0	57.5	5.0	11.5	56.0	
a • •	24.6	20	19.1	47.8	45.9	6.7	6.8	84.0	
Series 3,	20.9	25	19.6	49.6	41.9	8.1	5.2	92.0	
75 days	16.4	27	18.3	49.1	41.2	7.3	5.6	100.0	
	10.4	30	19.2	44.8	40.2	7.4	5.4	100.0	

measured (Table 3). Final stands were expressed as percentages of the original plantings. Representative sets of seedlings from both series were photographed (Fig. 2).

Post-emergence losses in both series were correlated with temperature, as was emergence reduction in Series 1. Most rapid and greatest losses

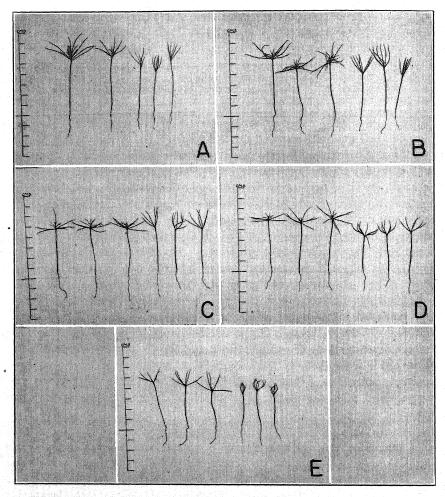


Fig. 2. Representative seedlings of *Pinus resinosa* grown in temperature chambers: A. Chamber I, 31.5° C.; B. Chamber II, 24.6° C.; C. Chamber III, 20.9° C.; D. Chamber IV, 16.4° C.; E. Chamber V, 10.4° C. The 3 larger seedlings on the left in each set are from Series 3 (75 days duration) and those on the right from Series 2 (45 days). Scale in centimeters.

occurred in the warmest chamber; with decreasing temperatures, total losses became less and seedlings were attacked less rapidly. In the same experiment, variations in temperature affected the development of the host. The most rapid growth of tops, as expressed by elongation of hypocotyls and development of leaves, occurred in direct relation to increasing temperatures

(Table 3 and figure 2). The "warm" plants, however, had weaker stems and less pigment than the "cold" ones. The characteristic increasing succulence with rise in temperature was further reflected in the ratios of green to dry weight of the seedlings. The ratios were higher at the higher temperatures.

In both series, final stands of controls were reduced at the higher temperatures. Since some dead seedlings from these controls were infected by contaminating organisms and others were free of infection, it was not possible to ascertain whether the total reduction was due to infection by these contaminants or to lethal effects of the high temperatures. If the former was the case, then unquestionably the higher temperatures predisposed the control seedlings to invasion.

Temperature effects on the growth of Fusarium oxysporum were tested in Petri dishes of potato-dextrose agar, adjusted to a final pH of 6 and incu-

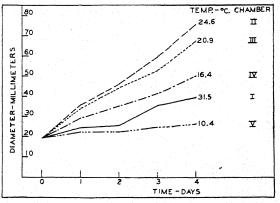


Fig. 3. Effects of several levels of temperature on the growth of Fusarium oxysporum on potato-dextrose agar.

bated 24 hours at room temperature before distribution to the chambers. The 5 dishes in each chamber were covered by continuously moistened cloth to equalize as far as possible the humidities at the different temperature levels. All the chambers were given normal exposure to light in order to subject the fungi to the same conditions which would prevail during the inoculation tests, although less fluctuating temperature equilibria could have been obtained in darkness. Colony diameter was measured after each 24-hour period for four days. The daily average diameters at each level of temperature are summarized in figure 3.

The fungus grew over the entire temperature range. Both in daily-increment and total-growth measurements, its maximum growth was at approximately 25° C., growth was slight at 31.5° C. and minimum development was at 10.4° C. The optimum was generally comparable with that of 25°-30° C. reported for the growth in pure culture of *Fusarium oxysporum* isolated from potato-wilt by Goss (5), although he demonstrated that the value varied with the substrate.

SUNLIGHT

The relationship of light to the damping-off problem is an indirect one. Diurnal and seasonal variations in insolation are the main sources of the temperature and moisture fluctuations that occur in the field and affect seedling development (1). Furthermore, light affects growth and survival directly (3, 7, 15, 19, 20). Smith (21) pointed out that the heavy shading of seedlings retards growth and prolongs succulence in a manner that may contribute to damping-off losses. Since nursery practice commonly employs shading in seedling beds, further knowledge is essential on the direct relation of light to damping-off.

The effects of variations in light intensity on the damping-off of coniferous seedlings were studied in the greenhouse. Slotted screens provided varying degrees of illumination (Fig. 4). These were preferred to shading

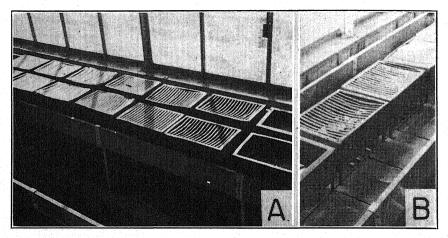


Fig. 4. A. Shading-screens providing varying degrees of radiation-intensity ranging from 0 per cent, upper left, to 100 per cent, lower right. B. View showing manner of supporting frames on legs.

cloths since lath shades are more common in nursery practice. They also provided conditions more closely resembling those under a natural canopy of vegetation, where plants are alternately exposed to high and low light intensities. The panes of the greenhouse were of ordinary clear glass, allowing most of the light to pass through.

The screens and supporting frames $(12\times18\times6)$ inches) were stiff corrugated paper and were painted on all surfaces with nonreflecting black paint. Each frame was supported on legs to raise its lower edge 3 inches above the surface of the bench and permit free circulation of air. At the same time the light-intercepting area was thus sufficiently elevated over the surfaces of the pots so that light intensity, measured with a photo-electric cell (Weston) was uniform at all locations in the seedling level. The width and spacing of the openings of the screens were planned to permit, after calibration with the photometer, various percentages of the light-intensity in the open frame:

75 per cent, 50 per cent, 25 per cent, 12 per cent, 6 per cent and 0 per cent. (See Fig. .4) Each shade frame covered two 7-inch pots.

Duplicate inoculations were made in autoclaved soil (3 parts loam, 2 sand, and 2 leaf-mold) with Fusarium oxysporum, F. vasinfectum and F. avenaceum, and each pair was tested under a single frame at each level of light-intensity. Fifty surface-sterilized seeds of Pinus resinosa and of Pinus sylvestris were sown in each inoculated pot after 5-days' incubation of the fungus. Post-emergence losses of seedlings through damping-off were recorded for 30 days. The tests were carried out on the south side of the greenhouse, the apertures of the screens arranged at right angles to the path of

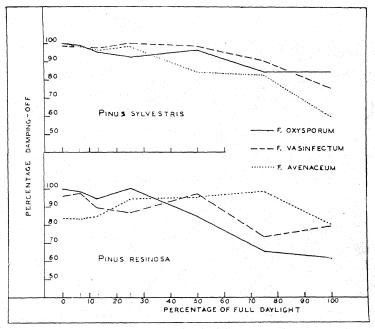


Fig. 5. Relation of light-intensity to damping-off of Pinus resinosa and Pinus sylvestris in sand cultures inoculated with Fusarium oxysporum, F. vasinfectum, and F. avenaceum. Data are based upon duplicate trials.

the sun. Damping-off losses in relation to light-intensities are represented in figure 5.

An additional set of uninoculated seedlings was tested, in duplicate pots, in the light-intensity range. The growth characteristics were measured 30 days after emergence (Table 4); and representative seedlings of both hosts grown under each light intensity are shown in figure 6. Succulence in both hosts, measured in terms of hypocotyl elongation and ratio of green to dry weight, increased with decreasing light-intensities. Root length was optimum for *Pinus resinosa* in 50 per cent and 75 per cent light and for *P. sylvestris* in 75 per cent light. The differences in root length, however, were probably an index to temperature variations due to the different degrees of insolation, although the practice of watering frequently during the day,

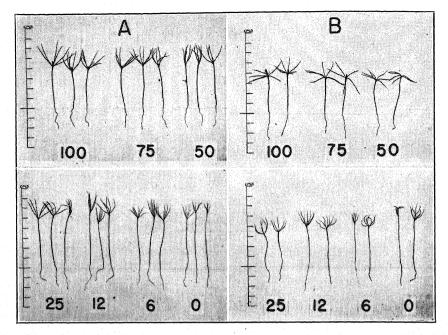


Fig. 6. Representative seedlings of *Pinus resinosa* (A) and *Pinus sylvestris* (B) grown for 30 days under different light-intensities (percentages of full light given numerically).

keeping soil-moisture content uniform in all pots, tended to keep temperatures from fluctuating too widely. Below-surface $(\frac{1}{4}-\frac{1}{2} \text{ inch})$ temperatures of the soils on the average varied from 18° to 23° C., and the maximum attained in any pot was 28° C. in pots exposed to full maximum solation.

The effects of similar light-intensities upon the growth of the fungi were measured. Lundegårdh (13) cited instances of the penetration of light rays

TABLE 4.—Relation of light intensity to the growth of Pinus resinosa and Pinus sylvestris in autoclaved-soil cultures. Duration of test 30 days following emergence

Plant	Per- centage of full	No. of seedlings				Average weight in milligrams		
	light	measured	Roots	Hypocotyls	Green	Dry	to dry weight	
	0	22	17.1	50.8	30.5	2,4	12.7	
	6	30	17.6	51.9	44.4	4.2	10.6	
Pinus	12	30	16.1	48.1	43.0	4.7	9.2	
resinosa	25	30	17.1	49.7	43.2	4.4	9.8	
1001110011	50	30	19.9	46.2	43.4	5.3	8.2	
	75	30	19.8	42.7	43.4	5.9	7.4	
	100	30	16.9	38.2	44.0	5.8	7.6	
	0	24	17.0	45.8	35.0	2.7	13.0	
	6	23	15.7	41.2	32.7	2.3	14.2	
Pinus	12	22	16.0	42.0	32.5	3.0	10.8	
sylvestris	25	24	15.1	37.2	27.5	3.1	8.9	
oguesuus	50	19	13.1	36.2	21.1	3.7	8.4	
	75	21	20.2	37.6	37.7	4.6	8.2	
	100	22	18.2	35.9	37.5	4.9	7.7	

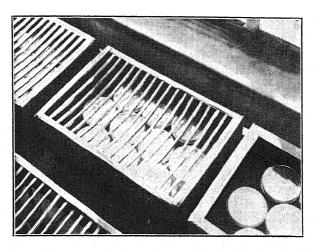


FIG. 7. Adaptation of screens for testing the relation of light intensity to the growth of fungi in Petri dishes.

below the surfaces of soils, measured in terms of the photosynthetic activities of soil algae growing at depths of several centimeters. Therefore, in the present experiments, the effects of light-intensity on the growth of the fungi probably bore some relation to the amount of disease they caused. Petri dishes of potato-dextrose agar were seeded with the fungi, five plates of each organism for each screen, and the plates were placed upon platforms at the same distance below the screens as were the soil-surfaces in the inoculation tests (Fig. 7). After exposure for 4 days, the average diameter of the colonies under the various screens was measured (Fig. 8). Growth of each fungus generally increased with reduction in light-intensity, agreeing with the observations of Harter (8), that the vegetative growth of various Fusaria was more abundant in the dark.

Under the conditions of the experiment, the effects on fungus growth of differences in temperature induced by different light-intensities could not

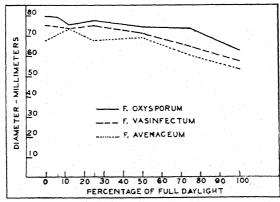


Fig. 8. Effects of light intensity on the growth of three species of Fusarium on potato-dextrose agar.

be wholly evaluated. Although greenhouse temperatures were maintained at 20–24° C., undoubtedly increasing insolation elevated temperatures beneath close-fitting Petri-dish covers. However, at no time were lethally high temperatures observed in any plates during the test, and periods of exposure of the plates to direct radiant heat, considered on a 24-hour basis, were relatively short.

DISCUSSION

Damping-off of *Pinus resinosa*, caused by *Fusarium oxysporum*, proved to be directly related to temperature. While high temperatures hastened the development of the host, its succulence also increased, so that its resistance to the fungus was lowered. Thus emergence and post-emergence losses were highest at temperatures beyond the optimum for fungus development. However, on the whole, the variation in losses in relation to temperature appeared to depend upon its effect simultaneously upon both host and pathogen. Increase in losses with mounting temperatures showed the same general progression with time in experiments differing in amount of inoculum, thus indicating a host response. On the other hand, the differences in the relative positions of the curves of losses between "heavy" and "light" inoculations, suggested a relation between the virulence of the fungus and the particular temperature conditions.

An important fact demonstrated by these experiments is the apparent existence of a critical temperature below which the host is able to grow and escape infection in infected soil. Roth (18) also described damage caused by Fusarium species only at high temperatures at which Pythium and Rhizoctonia were relatively harmless. In the present experiments, in both light and heavy inoculations of Pinus resinosa seedlings grown at an average temperature of approximately 10° C., losses were negligible. This fact showed a striking parallelism with other Fusarium wilts whose reactions to soil temperatures have been studied (11). These reports have also established minimum temperatures below which the hosts have grown fairly well and have escaped serious infections: for example, tomato wilt, 19° C.; cabbage yellows, 17° C.; flax wilt, 14° C. This phenomenon obviously is directly related to the thermophilic nature of the Fusaria generally, as has already been mentioned. The lowest temperature established in the apparatus of the present experiments, 10° C., probably did not represent the actual critical point. At an average of 16° C., the next highest temperature available, losses were low during the 75 days following a light inoculation of the fungus, and relatively high 45 days after a heavy inoculation. critical point was probably between 10° and 16° C.

The influence of light upon damping-off was related to its effect upon the host and the pathogen. With increasing shade, both the succulence of the host and the growth of the fungi became greater, and both apparently contributed to increasing damping-off losses. These results could be distinguished from any possibly caused by secondary effects of variations in the insolation. Two factors which vary primarily in the field with the amount

of sunlight are temperature and moisture. In these experiments, moisture variation was minimized through the practice of frequent and uniform watering. The abundant moisture further served to check rising temperatures with increasing insolation. Since maximum temperature variations were recorded for non-shaded pots, if the light experiments were being mainly influenced by secondary temperature variations, then the greatest losses could be expected in the unshaded pots. However, the results indicated that the converse was true, best stands being obtained in full light. This indicated that light conditions were affecting the results independently of secondary temperature effects. In the field, however, unquestionably beneficial effects of full light are lost. Moisture control becomes more difficult with higher insolation, and the higher rate of evaporation which results causes the soil to dry out more rapidly. This, in turn, permits higher soil temperatures and increases possibilities of fungus attack.

SUMMARY

The relation of temperature variations to damping-off and to the growth of the host and pathogen was tested in control equipment.

Optimum growth of Fusarium oxysporum was favored by a temperature of approximately 25° C. Emergence of Pinus resinosa was reduced lethally by some higher temperatures in the range tested, whereas its rate was slowed down by the lower levels. In post-emergent development, rapidity of growth and succulence of Pinus resinosa increased with rising temperatures.

Increase in emergence losses and post-emergence damping-off of *Pinus resinosa* was directly correlated with increasing temperatures. Both types of losses were relatively negligible at an average temperature of 10° C., regardless of the amount of the inoculum used. This suggests the existence of a critical temperature below which *Pinus resinosa* may grow and escape infection by *Fusarium oxysporum*, an organism which otherwise causes relatively high losses.

Damping-off losses of *Pinus resinosa* and *Pinus sylvestris* were in direct relation to decreasing light-intensities, maximum final stands being obtained in full light. This condition was correlated with the increase in succulence of the hosts and in growth of three Fusarium lines used in the inoculations, with decreasing light-intensities.

BOTANICAL LABORATORY,
UNIVERSITY OF PENNSYLVANIA,
PHHADELPHIA, PA.

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AN APPARATUS FOR THE GROWTH OF PLANTS UNDER CONTROLLED TEMPERATURE LEVELS

HOWARD TINT

(Accepted for publication January 15, 1945)

INTRODUCTION

Many descriptions have been published of control chambers suitable for experiments on the relation of temperature to plant growth. 1, 2, 3 For the most part, however, the construction and maintenance costs of apparatus limit its general use to relatively few institutions. While such equipment has permitted plant studies under a wide range of conditions controlled by dependable instruments, some problems may be explored in equipment just as adequate for the particular purpose for which it is designed, at relatively much lower expense.

Most apparatus permitting temperature control has been constructed according to the incubator principle, providing single temperatures which are constantly maintained. However, this is in effect not comparable with natural conditions, where temperatures change according to seasonal conditions and fluctuate daily within seasonal levels through the influence of solar radiation. In order to study the temperature relations of plant disease, for example, and to plan experiments comparable to field conditions and upon which predictions and recommendations for nursery practice may be based, temperatures controlled at various levels should be permitted to fluctuate in a similar manner.

In the course of an investigation of the temperature relations of the Fusarium damping-off disease of coniferous seedlings, the writer4 constructed and used an apparatus which successfully met these requirements and also embodied certain elements of design which made it adaptable to diverse problems in plant physiology, plant pathology, agronomy, etc.

DESCRIPTION OF APPARATUS

The apparatus, in its basic form, is a rectangular, insulated chamber, divided into compartments and bearing a heating unit at one end and a refrigeration unit at the other. Air circulates freely among the compartments, and in operation an air-temperature gradient is thus established over the length of the chamber, ranging from warmest in the compartment adjacent to the heater to coldest at the refrigerator end. Upon exposure of the interiors of the compartments to normal daylight, through glass tops, the

¹ Crocker, W. Organization, equipment, dedication. Contr. Boyce Thompson Inst. 1: 9-52. 1925.

² Davis, H. R., and D. R. Hoagland. An apparatus for the growth of plants in a controlled environment. Plant Physiol. 3: 277-292. 1928.

³ Johnson, J. Constant temperature and humidity chambers. Phytopath. 18: 227-

⁴ Tint, H. Studies in Fusarium damping-off of conifers. III. Relation of temperature and sunlight to the pathogenicity of Fusarium. Phytopath. 35: 498-510. 1945

temperature in each chamber then fluctuates about a different level, the greatest deviation from each mean temperature taking place during periods of maximum insolation and the least during periods of darkness.

Details of the construction are in figure 1. The chamber is built of three-fourths-inch white pine lumber, the bottom and four sides being double-walled, with a dead air-space packed with sawdust between. Half-inch ply-wood panels, inserted into grooves on the inner sides of the chamber, divide it into five compartments, which are covered by panes of glass with adjoining edges made smooth for close-fitting. The edges of the apparatus upon which the covers rest are further insulated with strips of felt. Additional compartments at both ends contain the heating and cooling units. These

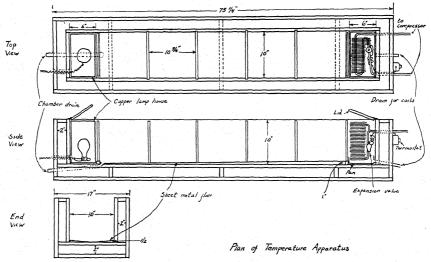


Fig. 1. Working drawings of elevations of the control apparatus.

spaces are separated from the interior of the chamber by additional panels and are covered by wooden doors inserted on the inner edges of the apparatus by hinges (Fig. 2, C, E). All panels are shortened at the top by one-quarter inch to permit free passage of air between adjacent compartments. In addition, the panels separating the spaces containing the heater and refrigerator units are also similarly shortened on the sides. These baffles thus permit free conduction of cold or warm air into the apparatus, at the same time preventing excessive radiation of heat into the chamber adjacent to the heater and freezing of solutions subsequently placed in close proximity to the refrigerator coils. Some panels bear holes permitting additional circulation of air (Fig. 2, B), which tends to space more evenly the temperature increments in the intervening compartments between the two extremes established at the ends.

Galvanized iron on the floor of the apparatus provides a graded surface in two directions (Fig. 1): a one-inch grade over the entire length and a half-inch slope from the edges to the center. Water drains towards an outlet at one end of the chamber (Fig. 2, C, D). A drip pan and drain are also provided for the compartment containing the refrigeration coils (Fig. 1). Open seams within the chamber are caulked with putty to prevent the entrance of water into the sawdust-filled air space. All interior surfaces are painted with white enamel, for reflection of light within the compartments.

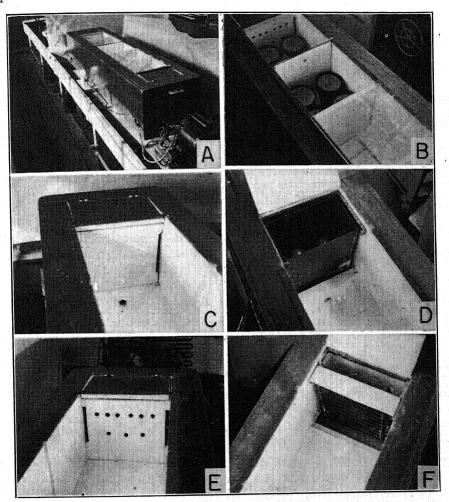


Fig. 2. A. View of entire apparatus. B. Enlarged view of equipment showing manner of disposition of pots of plants in the chambers. The glass cover of the central chamber has been removed. In the lower chamber, the manner of supporting pots on the sloping floor is illustrated. C. Warm end of chamber with lid and baffle in place. D. Same view as C with lid raised and cover of lamp house removed. The aperture for chamber-drain may be seen. E. Cold end of chamber, lid and baffle in place. F. Same view as E with refrigerator coil exposed.

Heat is supplied by electric bulbs housed in a copper container (Fig. 2, D), and the degree is controlled by varying the wattage of the bulbs. For the continuous and uniform supply of heat, the bulbs remain lighted for the duration of an experiment. Since ordinarily they are not visible, it is con-

venient to install in the exterior heater circuit an additional bulb in series (Fig. 2, A). A failure of this bulb to light indicates that the heating bulbs are inoperative.

Cold air is supplied by a small refrigeration machine equipped with thermostatic control, taken from a home-model refrigerator. The normal freezing coil is replaced by a double-pass fin-coil (Fig. 2, F) with an expansion valve control. The coil maintains low temperatures in the adjacent compartment (approximately 8° C. if desired) against the warm air coming from the other end of the apparatus.

The limits of the temperature range that may be established are thus conditioned by the relative degrees of heat and cold which are applied at the ends, and the spacing of the temperatures between these extremes in the intervening compartments is established experimentally by regulating the area through which air may circulate between adjacent chambers.

TEMPERATURE RECORDING

The actual temperatures in each of the chambers, in a 7-day test run with heat from a 20-watt bulb and a specific cold-setting, are shown in figure 3.

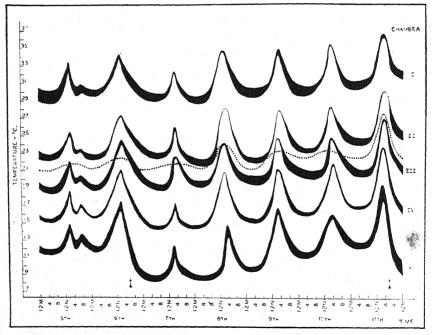


Fig. 3. Temperature record illustrating the levels of temperature secured within the air-control chambers over a period of one week, and the manner in which variation under the influence of insolation took place. Arrows indicate times of defrosting of refrigerator coils. The dotted line records the mean daily temperatures of the greenhouse during this period.

The width of the curve lines indicates the variance between the readings of 2 thermometers, suspended with bulbs at opposite ends of each compartment. Observations were hourly during daylight, and values for hours of

darkness usually were interpolated. In several 24-hour watches, after temperatures reached equilibria in darkness, they remained practically constant until daylight.

Diurnal fluctuations of temperature took place at different levels in the various chambers. There was a slight lack of uniformity for the two coldest chambers, whose values became generally higher as ice was deposited upon the freezing coil. Little change, however, was evident for 48 hours following defrosting (indicated in Fig. 3 by arrows). Consequently, in practice, the coils were defrosted every other day.

The sensitivity to variations in light intensity is evident. Maximum rise in temperature in the various chambers occurred during greatest insolation, bright days showing higher peaks than cloudy days. The sensitivity to light is evident in the record for the 5th day (Fig. 3). The morning and early afternoon were cloudy and dull, causing relatively slight rises in the temperature levels. In the late afternoon, however, the sun appeared very brightly, and as a consequence a secondary temperature rise in all the chambers was evident.

The levels of temperature attained in the several chambers of the apparatus and their fluctuations are independent of the external greenhouse temperature, which was controlled during the test.

The mean temperatures maintained in each chamber for the two days following defrosting—6th day, 6 p.m. to 8th day, 6 p.m.—and their standard deviations were: I, $31.5^{\circ} \pm 1.3^{\circ}$ C.; II, $24.6^{\circ} \pm 1.6^{\circ}$ C.; III, $20.9^{\circ} \pm 1.4^{\circ}$ C.; IV, $16.4^{\circ} \pm 1.5^{\circ}$ C.; and V, $10.4^{\circ} \pm 1.6^{\circ}$ C. The standard error of any mean was 0.3° C.

COST AND ADAPTABILITY

The cost of the apparatus was comparatively low. Materials used in construction totaled approximately \$25.00, and the price of the fin-coil, expansion valve, thermostat, and other installation expenses totaled less than \$45.00. The compressor is relatively inexpensive, or else refrigeration may be furnished by other machines which often are standard equipment for many laboratories. Daily maintenance costs are almost negligible.

The equipment, as described, has been adequate for testing the relation of temperature to plant growth and disease,⁵ when the particular purpose was to maintain a temperature range, each level of which duplicated natural conditions by increasing within its range under insolation. For other purposes, extensive modifications are possible. Constant temperatures may be secured, either by the exclusion of light, or, if light is essential, by the construction of a cover containing sources of light which produce no appreciable heat, as for instance, fluorescent filament-bulbs. In addition, a rheostat and thermostat included in the heating circuit would provide a more easily controllable source of heat.

⁵ See footnote 4.

STIMMARY

An apparatus is described which permits the investigation of plant development simultaneously at several levels of temperature. The design of the equipment allows temperatures in separate compartments to fluctuate within their respective levels in a manner resembling the normal diurnal range of temperature in the field under the influence of varying degrees of insolation.

Details for the construction of the equipment are given. The cost of the materials is relatively low and subsequent maintenance expenditures are negligible.

BOTANICAL LABORATORY,
UNIVERSITY OF PENNSYLVANIA,
PHILADELPHIA, PA.

A PRELIMINARY REPORT ON FURTHER STUDIES OF PHYSIOLOGIC SPECIALIZATION IN USTILAGO HORDEI¹

T. F. YU AND C. T. FANG²

(Accepted for publication January 15, 1945)

INTRODUCTION

In a previous paper (3) was reported an investigation at Nanking University, which led to the discovery of the existence in China of 5 distinct physiologic races of covered smut (*Ustilago hordei* (Pers.) K. and S.) of barley. These races, numbered C-1 to C-5 inclusive, were differentiated on three varieties of barley. On account of the war which forced the removal of Nanking University to Szechwan, the smut material was unfortunately lost during transit. The study was resumed in Kunming, Yunnan, in 1939. The results of the investigations from 1939 to 1943 are presented in this report. The literature on physiologic specialization of *Ustilago hordei* has been reviewed by Tapke (2) and Yu (3), and will not be reviewed again in this paper.

MATERIALS AND METHODS

Numerous collections of *Ustilago hordei* were obtained mostly from southwestern China. Fourteen varieties of barley were inoculated with most of these collections, and 4 barley varieties were selected as differential hosts. Nanking Nos. 368 and 373, which had been used in the previous study (3), were still retained as differential hosts. The two other varieties, Himalaya (C.I. 1312), and Excelsior (C.I. 1248), were received through the kindness of Dr. V. F. Tapke, United States Department of Agriculture. Finland, one of the differential hosts used in the previous investigation, was omitted because of its high degree of sterility when grown in this part of China.

The methods used in inoculation were those described by Tapke (1) in 1935. Inoculated seed was planted at about 2 grams in each of three 3-foot rows, with one or two replications. The smut percentages obtained were based on counts of the total number of heads per plot of 3 rows. Three infection classes were established: resistant class (R), 0–5 per cent smutted heads; intermediate class (I), 5.1–20 per cent smutted heads; and susceptible class (S), 20.1–100 per cent smutted heads.

¹ Paper No. 23 from the Division of Plant Pathology, The Institute of Agricultural Research, National Tsing Hua University.

PHYTOPATHOLOGY extends the courtesy of its journal pages to scientists in other countries who are persevering in research under difficult wartime conditions and are temporarily deprived of the opportunity for membership in the American Phytopathological Society.

²The writers take pleasure in acknowledging their indebtedness to Prof. F. L. Tai for the stimulating encouragement during the progress of the investigation; to Messrs. H. R. Wang and S. Y. Yin for assistance in field experiments; and to Drs. L. Ling, Szechwan, and C. S. Wang, Honan, for smut material.

TABLE 1.—Percentages of smutted heads obtained in 5 varieties of barley inoculated with 9 physiologic races of Ustilago hordei at Kunming, Yunnan, China, 1941-1943

Name of	Year		Physi	ologic	race ni	ımber	of Ust	ilago 1	hordei	
differential host	tested	1	2	3	4	5	6	7	8	9
The second section of the second		Pct.a	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Himalaya (C.I. 1312)	1941	0.0	0.0	11.7	0.0	0.0	5.2		0.0	•••••
,	1942	1.0	0.0	13.8	0.0	0.0	6.4	7.1	4.6	7.1
	1943	0.0	0.0	7.8	0.0	0.0	6.4	7.1	13.8	4.8
	Av.	0.3	0.0	11.1	0.0	0.0	6.0	7.1	9.2	6.0
Nanking No. 368	1941	2.1	0.0	2.3	4.0	0.0	0.0	0.0	9.7	7.5
Tranking 170. 000	1942	0.0	0.0	0.6	4.0	0.0	3.6	0.0	6.7	4.8
	1943	2.0	0.0	1.8	8.8	0.0	3.0	0.9	10.5	5.1
	Av.	1.4	0.0	1.6	5.6	0.0	2.2	0.3	9.0	5.8
Nanking No. 373	1941	0.0	0.0	6.3	0.0	11.0	18.6	9.5	8.7	18.6
8 2.01	1942	0.0	0.0	0.0	6.3	16.6	13.6	5.8	10.1	27.9
	1943	0.0	0.0	0.0	0.0	14.8	8.2	12.3	11.3	20.2
	Av.	0.0	0.0	2,1	2.1	14.1	13.5	9.2	10.0	22.2
Excelsior (C.I. 1248)	1941	5.4	13.7	0.0	0.0	0.0		24.5	3.6	10.0
(3	1942	6.0	10.7	0.0	0.0	1.5	11.8	21.8	5.1	10.4
	1943	3.6	8.5	4.2	0.0	0.0	12.0	26.1	7.0	13.4
	Av.	5.0	11.0	1.4	0.0	0.5	11.9	24.1	5.2	11.3
Local hulledb	1941	39.4	54.6	28.0	47.6	27.1	29.6	28.1	42.9	55.0
The second secon	1942	35.3	52.7	38.6	43.1	50.2	31.4	40.5	47.3	33.7
	1943	40.6	37.2	29.1	11.1	36.3	40.6	49.9	25.6	43.8
	Av.	38.4	48.2	31.9	33.9	37.9	33.9	39.5	38.6	44.2

² Average percentages of smutted heads of 2 or 3 plots.

RESULTS

The average percentages of smutted heads produced by the races on the differential hosts in 1941, 1942, and 1943 are recorded in table 1 and the reactions that serve to differentiate the races of *Ustilago hordei* are presented in table 2.

 ${\bf TABLE~2.} \\ -Relative~susceptibility~of~4~differential~hosts~to~9~physiologic~races~of~Ustilago~hordei$

Differential heat	Reaction ^a to each physiologic race								
Differential host	. 1	2	3	4	5	6	7	8	9
Himalaya (C.I. 1312) Nanking No. 368 Nanking No. 373 Excelsior (C.I. 1248)	$\frac{R}{R}$	R R R I	I R R R	R I R R	R R I R	I R I I	I R I S	I I I	I S I

^{*} R = 0-5 per cent; I = 5.1-20 per cent; and S = 20.1-100 per cent infection.

There are at least 9 distinct physiologic races which can be recognized by their effects on 4 varieties of barley (Table 1). The differences in the infection capabilities are shown more clearly in the following analytical key:

b Susceptible check variety.

Analytical key for identification of 9 physiologic races of Ustilago hordei

Nanking No. 373 resistant	Race No.
Nanking No. 368 resistant	
Himalaya resistant	
Excelsior resistant	1
Excelsior intermediate	2
Himalaya intermediate	3
Nanking No. 368 intermediate	4
Nanking No. 373 intermediate	
Nanking No. 368 resistant	
Himalaya resistant	5
Himalaya intermediate	
Excelsior intermediate	6
Excelsior susceptible	7
Nanking No. 368 intermediate	8
Nanking No. 373 susceptible	9

Race 1 is by far the weakest in pathogenicity. In fact, all of the 14 varieties of barley are resistant to it. Races 4, 8, and 9 produced an intermediate reaction on Nanking No. 368, a hull-less barley introduced from Japan. It has been known as highly resistant to covered smut of barley at many places in China.

Of a total of 84 smut collections, many have been tested for only one or two years and thus data do not permit a detailed analysis of the prevalence and distribution of the races in China. Nevertheless races 1 and 2 evidently are widely distributed in China. The former occurred in 33 and the latter in 19 of the 84 collections.

Hanchen and Trebi barleys, which have been included in the 14 hosts, were highly resistant to almost all the smut collections. They had, however, a few smutted heads when inoculated with certain smut collections. When smut spores from either Hanchen or Trebi were used to inoculate these same hosts respectively an increase in smut resulted. This indicates that some of the smut collections were a mixture of two or more races and that there are possibilities of isolating a race or races to which either Hanchen or Trebi, or both, will not be resistant.

SUMMARY

Eighty-four collections of covered smut of barley were obtained from many places in China. Fourteen varieties of barley were inoculated with most of these collections.

Nine physiologic races of *Ustilago hordei* can be recognized by their parasitic behavior on four varieties of barley. They have been designated races 1 to 9.

Races 1 and 2 were collected more often than the others. But detailed analysis of the distribution and prevalence of physiologic races of *Ustilago hordei* in China cannot be made until more records are available.

It is likely that there are other physiologic races which could be recognized if more collections were made and more differential hosts inoculated.

INSTITUTE OF AGRICULTURAL RESEARCH,

TSING HUA UNIVERSITY.

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APPLICATION OF PENICILLIN TO CROWN GALL

J. G. BROWN AND ALICE M. BOYLE

(Accepted for publication February 15, 1945)

The action of crude penicillin on crown gall has resulted in the destruction (Fig. 1, B, C) of the treated galls. The results are noteworthy because the cause of crown gall, Agrobacterium (Phytomonas) tumefaciens, is a Gram-negative bacterium and, as such, member of a group regarded (1, 5, 6) as resistant to the action of penicillin. They also are interesting because crown gall, the disease concerned, is commonly regarded as incurable, although a few chemicals have been reported (2, 3, 4) as curative when properly applied.

The crude penicillin was produced in this laboratory from an improved strain of *Penicillium notatum* obtained from the Northern Regional Laboratory, Peoria, Illinois. The fungus was grown in a simple apparatus (Fig. 2) that can be sterilized in the autoclave.

Media used for growing *Penicillium* were modified Czapek-Dox (1) and corn-grain juice. The latter was prepared by soaking and afterwards simmering for a half-hour 50 g. dry weight of corn grains per l. of tap water. The media were steam-sterilized and filtered before use. As a further precaution, crude penicillin from the cultures was always filtered through a Chamberland or a Mandler candle. Thus made, the cost of the crude penicillin is approximately two cents per quart.

Soft galls were developed on plants of Bryophyllum as a result of inoculations with a pure culture of Agrobacterium tumefaciens. Crude penicillin that assayed 6 Oxford units was injected hypodermically into galls on young plants and into the stems of the host just below the galls in older plants. Growth of galls on young plants was checked, but the galls were not killed by one treatment by injection (Fig. 1, B, c) although the resulting necrosis was extensive in some cases. In larger galls likewise treated growth was checked just above the needle punctures, which accentuated the irregularity of gall surface.

The galls were then wrapped with cotton wool soaked in crude penicillin. That method of application resulted in retarded growth and browning of the small elevations on the surface of the galls; elsewhere there appeared to be lack of penetration of the penicillin into the gall. Numerous punctures of the galls, made by thrusting a sterile needle through the cotton wrapping, were soon followed by the death (Fig. 1, C, e, f) of the galls. The parenchymatous tissues became brown, vascular strands remained colorless, and the internal necrosis was limited to the tissues of the gall. However, some superficial injury resulted in normal parts of the stem that were in contact with the overlapping cotton wrapping (Fig. 1, C, e, f). Controls were unaffected.

Penicillin has been reported (7) as bactericidal for the crown-gall bacterium in vitro. Waksman and co-workers say that the bacterium "may

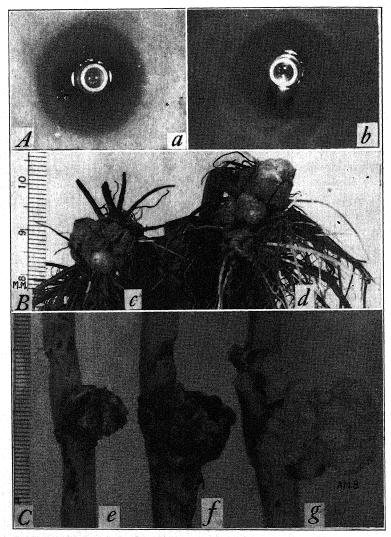


Fig. 1. A. Effect of crude penicillin in vitro, a, on Agrobacterium (Phytomonas) tumefaciens; b, on Staphylococcus aureus; cultures same age, subjected to penicillin at same time, from same flask, run simultaneously at same temperature. B, C. Effect of crude penicillin in vivo on young gall (B) and on older galls (C) of Bryophyllum; c, most of gall killed as a result of 4 simultaneous hypodermic injections of penicillin but with diffusion of chemical apparently incomplete; d, control galls that were approximately the same size as galls in c at time of injection; e and f, older galls killed by crude penicillin applied through cotton wrapping; e, longer exposed to air, dry and brown; f, drying and browning; g, control. All three galls same age and approximately same size before treatment.

be considered as fairly resistant when compared with the sensitivity to penicillin of the Gram-positive bacteria (Staphylococcus aureus and Bacillus

subtilis)." In our tests in vitro, limited to the cup-method, little, if any, difference in sensitivity to penicillin was found when Agrobacterium tumefaciens was compared with Staphylococcus aureus. Perhaps neither the tests reported by Waksman et al. nor our tests give a true picture of the comparative sensitivity of the two bacteria. In the former paper it is stated that the test cultures were run at 37° C, the optimum temperature for Staphylococcus aureus but the maximum temperature (9° C-12° C above the optimum) for Agrobacterium tumefaciens, considerably to the advantage of the former organism. Our cultures were run at room temperature, 22° C-26° C, below the optimum for Staphylococcus aureus and partly below the optimum for Agrobacterium tumefaciens.

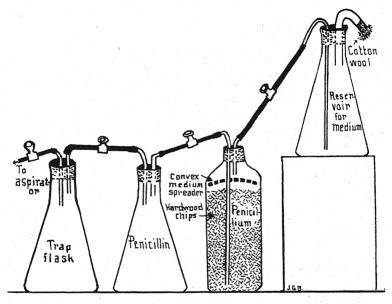


Fig. 2. Diagram of apparatus for producing crude penicillin.

In conclusion it may be remarked that crown gall, practically world-wide in distribution, is particularly serious in the Southwest. The alkaline reaction of the soil, mild climate, and the practice of irrigation favor the development and spread of the bacterium that causes the disease, and the heavy transpiration in a semi-arid atmosphere adversely affects the host. Entire orchards have been destroyed by crown gall. In clean soil the galls usually first appear on the crowns of plants from which the infection later spreads to the roots. The primary source of infection usually can be traced to the nursery. Crude penicillin should prove valuable in combating crown gall of trees and shrubs in which galls are limited to the crown. Possibly it may also be useful in budding and grafting operations in nurseries in which crown gall so frequently has its inception.

University of Arizona, Tucson, Arizona.

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THE MODE OF INFECTION AND THE INCUBATION PERIOD IN THE STEM SMUT OF GRASSES, USTILAGO SPEGAZZINII (U. HYPODYTES)¹

GEORGE W. FISCHER

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INTRODUCTION

Stem smut caused by Ustilago spegazzinii Hirsch. and U. spegazzinii var. agrestis G. W. Fisch. and Hirsch. (until recently (9) considered as U. hypodutes (Schlecht.) Fr.) is a common disease of many grasses in many parts of the world. Because of its effect on the host plants and the fact that it often attains epiphytotic proportions, the disease has attracted world-wide attention. Many species of grasses, in various genera and tribes have been listed as hosts (3, 5, 6, 8, 11, 13). It has been shown recently, however (9), that some of these records refer to other stem smuts similar in character, but caused by U. halophila Speg., U. nummularia Speg., and U. williamsii (Griff.) Lavrov, species which are morphologically distinct from U. spegazzinii and the variety agrestis. The morphological modifications of the host caused by stem smut have been especially studied by Feucht (7) and Bond (2), showing that parasitic infection results in the development of sterile culms bearing an excessive number of internodes and leaves in place of an inflorescence. Boss (4) and also Kharbush (10) studied the cytology of the fungus. Berkeley (1) called attention to the perennation of the fungus in the perennial tissues of the host plants, and this has been noted frequently since that time. The general biology of the stem smut fungus on Elymus arenarius L. was carefully investigated by Bornhövd (3), including inoculation experiments. She tried seedling infection, blossom infection, and infection of the young shoots, all with negative results. Bond (2) theorized that "infection takes place at the seedling stage rather than later." However, he was unable to germinate the spores of the smut and hence could not prove his theory.

Thus it is seen that in spite of much attention, study, and observation by many persons, the mode of infection in this common stem smut of grasses has thus far remained undiscovered. It is the purpose of this paper to present experimental and observational data which indicate the method of infection and the incubation period of the stem smut of grasses, caused by *Ustilago spegazzinii* and the variety agrestis.

OBSERVATIONAL DATA

In 1939 stem smut appeared spontaneously in severe proportions in a large plot of slender wheatgrass, Agropyron trachycaulum (Link) Malte,

¹ Grass disease investigations of the United States Department of Agriculture, Agricultural Research Administration, Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Forage Crops and Diseases, in cooperation with the Soil Conservation Service, Division of Nurseries, and the Washington Agricultural Experiment Station, Pullman, Wash. Published with the approval of the director as Scientific Paper No. 608.

and another of crested wheatgrass, A. cristatum (L.) Gaertner, in the Bureau of Plant Industry grass breeding nursery, Pullman, Washington. The plot of slender wheatgrass consisted of 1256 spaced, individual plants, representing the progenies of 248 selfed head selections. The plot of crested wheatgrass consisted of 690 rows, each row resulting from the seed of a single plant selection. Both were seeded in the spring of 1937, and headed out during the summer.

Table 1 shows the incidence of stem smut in the slender wheatgrass, 1937–1940 (after which the plot was destroyed), and table 2 shows the data for the crested wheatgrass, 1937–1943.

No stem smut appeared until the third growing season, in both the slender and crested wheatgrass, and the amount increased sharply in subsequent seasons. These observations are substantiated by similar observations in the Strain Trial Planting of Forage Grasses, in cooperation with the Soil Conservation Service, Pullman, Wash. These grasses were seeded in the

TABLE 1.—Comparative incidence, on a plant basis, of stem smut (Ustilago spegazzinii var. agrestis) in the same plot of slender wheatgrass during 1937-40

Yeara	Plants ^b in plot	Smutted	Smutted
	Number	Number	Per cent
1937	1256	. 0 :	0.00
1938	1256	0	0.00
1939	1241	332	26.75
1940	1234	427	34.60

^a The plants were transplanted (spaced) to the field in the spring in 1937. They beaded out the same season, and, of course, every season since.

^b The 1256 plants in the plot represented the progenies of 248 selfed head selections, of which 129 selections had more or less smut.

spring of 1939. The first stem smut appeared in 1941, the third season after seeding, with a tremendous increase in 1942, 28 and 70 rows, respectively.

This same observation has been made in the extensive observational row nurseries and the seed increase plots of the Soil Conservation Service, at Pullman, Washington. The first of these nurseries was seeded in the spring of 1936, and a new nursery has been planted each spring since. In these several nurseries and in the seed increase plots, no stem smut (due to *Ustilago spegazzinii* or the variety *agrestis*)² appeared before the third growing season.

In addition to the observational data obtained from grass nurseries and seed increase plots, the same minimum three-season interim between time of planting and the first expression of stem smut has been noted in fields of crested wheatgrass in the vicinity of Pullman, Washington. Many fields of this grass have been inspected for stem smut and in every case where it was possible to ascertain the age of the stand, stem smut was found only in fields that were in the third or subsequent heading.

² Stem smut due to *Ustilago williamsii* (9) was observed and collected on *Stipa* and *Oryzopsis* in earlier headings, indicating that this stem smut species has a different life history.

TABLE 2.—Comparative incidence, on a row basis, of stem smut (Ustilago spegazzinii var. agrestis) in the same plot of crested wheatgrass during 1937-43%, b

No.	No. of							
Selection	rowsc	1939	1940	1941	1942	1943	smutted 1943	
							Per cen	
50	1	0	0	0	0	0	0	
53	$\overline{3}$	0	0	0	0	0.	0	
926	4	0	0	0	2	3	75	
1916	11	0	3	7	9	11.	100	
1917	20	4	17	19	20	20	100	
1918	10	3	9	9	10	9	90	
1919	12	4	12	12	11	12	100	
1920	13	0	8	9	11	11	85	
1921	12	0	8 5	10	$\frac{12}{7}$	12	100	
1922	15	$\frac{4}{6}$		5 19	$\begin{array}{c} 7 \\ 25 \end{array}$	$\frac{10}{27}$	67 75	
1923	$\begin{array}{c} 36 \\ 23 \end{array}$. 11	18 18	20	$\frac{25}{21}$	27 22	96	
1924	25 19	8	12	13	16	17	89	
$1925 \\ 1926$	9	Õ	4	6	7	8	89	
1928	5	0	5	5	5	5	100	
1929	7	0	4	$\frac{3}{4}$	5	7	100	
1931	18	0	$\overline{4}$	$\overline{4}$	5	8	44	
1932	$\frac{10}{17}$	3	10	11	13	15	88	
1933	4	ō	3	3	4	4	100	
1934	11	0	5	6	10	11	100	
1935	3	0	3	- 3	3	3	100	
1936	7	0	6	6	7	7	100	
1937	19	5	17	18	18	18	95	
1938	1	0	1	1	1	1	100	
1939	17	3	14	16	16	17	100	
1940	3	0	3	3	3	$\frac{3}{0}$	100 0	
1941	1	0	0	$egin{pmatrix} 0 \ 4 \end{bmatrix}$	0	6	100	
1942	6	0	2	1	5 1	2	25	
1944	8	0	$\frac{1}{2}$	$\frac{1}{2}$	3	4	44	
1945	9 7	0	4	6	5	7	100	
$1948 \\ 1950$	6	1	4	5	6	6	100	
1950	16	$\frac{1}{2}$	10	13	16	16	100	
1952	11	õ	7	10	11	11	100	
1954	$\overline{16}$. 2	14	15	16	16	100	
1955	8	0	3	3	6	7	88	
1956	10	2	6	7	9	9	90	
1957	7	1	4	3	$\frac{2}{2}$	6	86 67	
1959	9	0	0	0	1	6_1	100	
1960	1	0	1	1 0	0	1	100	
1961	1	0	$\begin{smallmatrix}0\\1\end{smallmatrix}$	1	2	2	33	
1962	6 1	0	1	0	ĩ	ī	100	
1963 1964	1	0	0	ő	ō	1	100	
1967	$\overset{\mathtt{1}}{2}$	1	2	2	2	2	100	
1968	10	3	10	10	10	10	100	
1969	9	0	7	7	8 1	8	89	
1970	10	4	5	6	6	8	80	
1972	6	4	5	6	6	8	100	
1974	2	î	2	2 5	2	2	100	
1975	5	$\frac{1}{2}$	5	5	5	5	100 100	
1976	4	4	4	4	$\frac{4}{3}$	4 3	100	
1977	3	2 7	2	$\begin{array}{c} 3 \\ 12 \end{array}$	15	16	84	
1978	19	7	11	12	15 4	5	100	
1981	5 8	$\frac{2}{2}$	4 6	3 6	7	8	100	
1983	8					ĭ	50	

TABLE 2.—(Continued)

No.	No. of		No. of rows having smut				
	rowsc	1939	1940	1941	1942	1943	smutted, 1943
							Per cent
1985	11	0	1 1	1	1	2	18
1986	$\frac{11}{24}$	7	18	20	23	24	100
1987	1	Ö	0	0	0	0	0
1989	$1\overline{5}$	Ŏ.		1	3	8	53
1990	6	1	3 5 1	6	6	6	100
1991	ĭ	$\tilde{0}$	1	1	1	1	100
1992	5	3	5	5	5	5	100
1994	13	ĭ	3	8	9	11	85
1995	8	ō	Ŏ	1	3	6	75
1997	14	6	10	13	13	14	100
1999	12	ĭ	5	10	11	11	92
2004	3	ī	2	2	0	2	67
2005	ĭ	ī	1	1	1	1	100
2006	9	6	7	$\bar{7}$	9	8	89
2007	$\overset{\circ}{2}$	Ö	i	1	2	2	100
2008	5	ĭ	$\overline{4}$	5	5	5	100
2012	7	ō	$\bar{1}$	5	5	7	100
2019	2	ŏ	ī	ī	2	2	100
2412	$2\overline{4}$	2	8	13	18	22	92
Lind			· ·				
selection	14	6	9	9	12	13	93
101	î	ŏ	Õ	0	1	1	100
102	ī	ŏ	i	ĭ	î	ĩ	100
103	ī	ŏ	0	ī	ī	ĩ	100
104-6	ī	ŏ	0 .	î	î	ĩ	100
Totals	690	124	402	460	533	595	
Percentage smut	********	18	58	67	77	86	

^a The plants were transplanted from the greenhouse to the field in the spring of 1937 but were not space-planted. They headed for the first time during the summer of 1937, and since no smut appeared in 1937 and 1938, these years are omitted from the table.

^b This table was prepared by E. J. Kreizinger, formerly Assistant Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering.

c Each row resulted from seed taken from a single plant selection.

These data constitute strong circumstantial evidence that *Ustilago spegazzinii* and *U. spegazzinii* var. agrestis have a unique life history. It seems very probable that the incubation period is at least two years, or, more exactly, that symptoms are not expressed until the third heading after infection, or even later. Also, the yearly increase of stem smut in the stands of slender and crested wheatgrass strongly indicates a plant-to-plant spread of this disease.

EXPERIMENTAL DATA

Results of tests with naturally inoculated seed.—From the plot of slender wheatgrass in which stem smut was severe in 1939, seed was collected during the same season as follows: (1) from smut-free culms on otherwise smutted plants; (2) from smut-free plants with heavily-smutted plants to the windward (prevailing S.W. winds); (3) from smut-free plants entirely surrounded by smutted plants; and, (4) from healthy plants surrounded by

healthy plants. This seed was planted in the greenhouse in wood veneer plant bands and the seedlings transplanted to the field in the spring of 1940. The plants headed well in 1940 and in three successive seasons, but no stem smut appeared in any of them.

Results of artificial inoculations.—During the summer of 1939, mature plants and blossoms were inoculated with Ustilago spegazzinii var. agrestis and seed was inoculated by the vacuum method. The inoculation of mature plants was as follows: The plants were clipped back (in August, 1939) to within 5 or 6 inches above the ground. They were then sprayed with a spore suspension and covered with several layers of wet burlap for 48 hours. Crested wheatgrass, slender wheatgrass, and Canada wild rye (Elymus

TABLE 3.—Results of blossom, seed, and plant inoculations of Agropyron trachycaulum, A. cristatum, and Elymus canadensis with stem smut, Ustilago spegazzinii var. agrestis. Blossom inoculations performed 1938, seed and plant inoculations, 1939

		eed ar					Plant	inocu	lations	ı		
Collection and source of inoculum	blossom inoculations, all species ²		Agropyron trachycaulum		Agropyron cristatum			Elymus canadensis				
	1940	1941	1942	1940	1941	1942	1940	1941	1942	1940	1941	1942
F-C Agropyron									-	market days and the	-	
repens	_b	-		_	+	+		+	+	_		+
F-D A. cris-												
tatum		_	_			+	. <u></u> -	_	+			+
F-E A. trachy-												
caulum				_		_	-	+	+	_	-	+
F-G Elymus					, w .							
condensatus	-	-	_	·	+	+		-	+	-		+
F-H Agropyron												
spicatum	_		-	-	-	+	- .	-	+			+
F-Î Elymus												
glaucus	· - ·	_	-		+	+		_	-		, . 	+

a Since all seed and blossom inoculation results were negative the data are presented in this condensed form.

b Only the incidence (+) or absence (-) of stem smut is recorded. Percentages of infection were not determined, but the amount varied from a trace in 1941 to heavy in 1942. For the sake of brevity the check rows are not included but these were maintained for all inoculations and were smut-free.

canadensis L.) were each inoculated with 6 collections of stem smut from different hosts. The inoculated plants headed well in 1940 and subsequent years. No stem smut appeared in 1940; in 1941, however, slight infection was noted in some of the rows of slender wheatgrass and crested wheatgrass. In the summer of 1942 stem smut was abundant in nearly all the rows of slender wheatgrass and crested wheatgrass, and in all the rows of the Canada wild rye. No smut occurred in the check rows at any time.

In the experiment involving blossom inoculation whole spikes were inoculated (during the summer of 1938) with suspensions of stem smut spores, using Moore's portable evacuation method (12). Attention was given to different stages represented in the development of the fertilized ovaries, from the time of pollination to the medium dough stage. The same grasses and

the same smut collections were used as in the plant inoculations. The seed from such inoculated spikes were planted in plant bands and the seedlings transplanted to the field in the spring of 1939. The plants headed well in 1939 and in subsequent years, but no smut appeared at any time.

Also in the spring of 1939, seed of the same grass species was inoculated with the same 6 stem smut collections by immersion in aqueous spore suspensions under partial vacuum. This inoculated seed was planted in the greenhouse and the resulting seedlings were later transplanted to the field. These also headed well in 1939 and in subsequent years, but no smut appeared.

Table 3 shows the success resulting from the plant inoculations and the failure with the seed and blossom inoculations. The results with the plant inoculations substantiate the field observations that stem smut occurs in the third or later heading after planting. The only exception to this that has thus far been encountered is seen in the appearance of a very slight amount of stem smut in Agropyron trachycaulum and A. cristatum in 1941, in the second heading after plant inoculation in 1939.

It appears that stem smut caused by *Ustilago spegazzinii* and the variety agrestis is not seed-borne, either through blossom infection or external seed contamination. Rather, it seems probable that meristematic regions of the plants are infected and that this infection is not expressed by sporulation before the second heading following infection, and usually not until the third and subsequent headings.

DISCUSSION

The foregoing experimental data are probably insufficiently exhaustive to establish that infection of the host by *Ustilago spegazzinii* and the var. agrestis occurs in the purely vegetative tissues of the host to the exclusion of other modes of infection. The experiments which seem to establish this important part of the life history need amplification. This is especially needed to determine whether or not some injury to the plants is necessary at the time of inoculation, and also to determine if susceptible plants of any age, from seedling to maturity, can be infected. However, the negative results of the seed and blossom inoculations are in support of the infection of vegetative tissues. Also in support of the latter, is a vast accumulation of observations that indicate the infection of vegetative parts of the host during the period of sporulation of the smut, and the lapse of at least 2 years between time of infection and the appearance of stem smut symptoms.

Observations of the incidence of stem smut in stands of crested wheatgrass, slender wheatgrass, *Elymus spp.*, *Poa spp.* and other grasses during the past several years show an alarming tendency for the disease to "build up" in established stands. This seems entirely in line with the demonstration of the ability of the stem smut organism to infect mature plants. It seems likely that there is a plant-to-plant spread, once the fungus becomes established in a field or other planting. If this should prove to be the case, that stem smut spreads from plant to plant, once established, then the presence of stem smut would certainly be a factor in determining how long to maintain a stand of a susceptible species, because stem smut certainly renders the affected grass unfit for hay and seed, and possibly for pasture also. The control of such a disease would be very difficult, with the only practical method lying in the use of resistant varieties. Very little resistance has been apparent, however, in the plantings and species that have been under observation. Some salvation may be found in the fact that stem smut has such a long incubation period, and in the fact that apparently the disease is not seed-borne. Thus it seems probable that plantings of susceptible grasses (and most of our locally better species are susceptible) could be maintained five years or more, unless there were a heavy initial infection.

The mode of infection in *Ustilago spegazzinii* and the var. agrestis, by the infection of vegetative tissues, is not new to the biology of the smut fungi, but apparently such a long incubation period is unique in this group of plant pathogens. There appears to be no record of any other smut fungus in which sporulation is delayed until at least the second and usually the third heading following infection, after which it is perennial in the perennial parts of the host. At present a plausible explanation for this phenomenon is entirely lacking.

If, as the data indicate, stem smut caused by *Ustilago spegazzinii* and the variety agrestis is not seed-borne, the question arises as to the source of infection in the many grass species which have recently been smutted in the Pullman area. In answer to this question an accusing finger may well be pointed at extensive natural stands of quack grass, Agropyron repens. Large patches of this grass, very heavily infected with stem smut, are so common locally that it seems quite possible that there could be a sufficiently heavy spore shower to infect other grasses over a wide area. However, it remains for cross-inoculation experiments to definitely prove or disprove this theory. Thus far, the infection of crested wheatgrass (A. cristatum), slender wheatgrass (A. trachycaulum), and Canada wild rye (Elymus canadensis) with stem smut from local A. repens is in support of the theory.

SUMMARY

- 1. The results are given of seed, blossom, and mature plant inoculations with *Ustilago spegazzinii* and *U. spegazzinii* var. agrestis (*U. hypodytes* (Schlect.) Fr.), in the Pullman, Washington, region.
- 2. Infection was obtained in Agropyron cristatum, A. trachycaulum, and Elymus canadensis, with stem smut spores from several species of Agropyron and Elymus only by inoculation of mature plants.
- 3. Seed and blossom inoculations gave only negative results. No smut appeared in several hundred plants of *Agropyron trachycaulum* grown from seed taken from partially infected plants and from plants more or less surrounded by heavily infected plants.

³ In table 2, are listed several selections of crested wheatgrass which appear to have a high degree of resistance to stem smut. These are being used by E. J. Kreizinger as a source of stem smut resistance in crested wheatgrass.

- 4. The period of incubation was at least 2 years, and more often 3 years. In the successful inoculation experiments a trace of stem smut appeared in the second heading following inoculation, but at the third heading it was abundant. In numerous observations of the incidence of stem smut in commercial plantings, seed increase plots, and nursery rows the stand in every case was in at least its third heading.
- 5. It is thought that the source of infection lies in the common local heavy infestations of stem smut in quack grass, Agropyron repens. Experimentally, stem smut was induced in A. cristatum, A. trachycaulum, and Elumus canadensis with spores from A. repens as inoculum.
- 6. It has been observed that stem smut, once established in a stand of a susceptible grass, tends to increase rapidly in successive years, thus indicating a plant-to-plant spread.
- 7. Control of such a disease promises to be difficult. Probably the most practicable method will be the use of resistant strains of forage grasses. It is suggested, also, that because of the long incubation period, stem smut will not appear until at least 3 years after a susceptible grass is planted and will probably not become much of a production factor for 2 or 3 years more (unless there is a heavy initial infection), after which it may be advisable to plow up the stand.

WASHINGTON AGRICULTURAL EXPERIMENT STATION. PULLMAN, WASHINGTON.

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THE REACTION OF 21 SPECIES IN THE CUCURBITACEAE TO ARTIFICIAL INFECTION WITH CANTALOUPE POWDERY MILDEW (ERYSIPHE CICHORACEARUM DC.)

THOMAS W. WHITAKER AND DEAN E. PRYOR2

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Powdery mildew (Erysiphe cichoracearum DC.) has been a serious menace to the cantaloupe industry in practically all of the producing regions of the southwestern States since 1926. In developing a plant breeding program to combat this parasite by means of disease-resistant varieties, it is important to know the reactions of other species in the Cucurbitaceae to artificial infection with the fungus. This information should indicate possible potential hosts in the native, as well as the cultivated, flora. It may perhaps furnish a valuable clue in pointing out those genera and species that carry genes for disease resistance and, hence, are of possible value for hybridization with Cucumis melo L., the species to which the cantaloupe and related melons belong.

While this survey was by no means extensive, it included representatives of many of the genera of the Cucurbitaceae that have been cultivated from time to time; also, with one exception, it included the genera of the Cucurbitaceae native in southern California.

METHODS

Plants grown in the greenhouse were artificially inoculated in a chamber designed for the purpose.3 Within 16 days reasonably precise readings could be made of the resistance of individual plants. For convenience in recording results, 5 categories based on the extent of mycelium growth and degree of sporulation on leaves, cotyledons, and stems were used: 0 indicated no macroscopically visible mycelium and 4 indicated abundant mycelial growth and conidial production (See Pryor and Whitaker4 for details regarding rating the amount of mildew).

RESULTS

The reactions of 21 species in 11 genera are recorded in table 1. Seven of the 21 species had a high level of resistance to cantaloupe powdery mildew: Citrullus vulgaris Schrad. (watermelon), Cucumis anguria L. (West Indian gherkin), Cyclanthera explodens Naud., C. pedata Schrad., Ecballium elaterium (L.) A. Rich. (squirting cucumber), Luffa acutangula (L.) Roxb. (dishrag gourd), and L. aegyptiaca Mill. (dishrag gourd).

Three native species were susceptible: Cucurbita foetidissima H.B.K.

¹ Geneticist, United States Department of Agriculture.
² Associate Plant Pathologist—on military leave.
³ Pryor, Dean E. The influence of vitamin B₁ on the development of cantaloupe powdery mildew. Phytopath. 32: 885-895. 1942.
⁴ Pryor, D. E., and Whitaker, T. W. The reaction of cantaloupe strains to powdery mildew. Phytopath. 32: 905-1004. 1942. mildew. Phytopath. 32: 995-1004. 1942.

TABLE 1.—The reaction of species in the Cucurbitaceae to artificial infection with cantaloupe powdery mildew

	No. plants		Mildew reaction ^b			
Species	tested		Leaves	Cotyledons	Stems	
Benincasa hispida (Thumb.) Cogn	21		0	0-2	0-4	
Bryonia dioica L.			0-2	0-4	0	
Citrullus vulgaris Schrad			0	0	0	
Cucumis melo L			0-4	0-4	0-4	
Cucumis sativus L.	100a		1-4	3-4	0-4	
Cucumis anguria L.	4		0	0	0-2	
Cucumis metuliferus E. Mey			4	1–2	4	
Cucurbita pepo L	100a		2-4	2-4	0-4	
Cucurbita moschata Duch	100a		1-4	2-4	0-4	
Cucurbita maxima Duch			0-4	2-4	0-4	
Cucurbita ficiolia Bouché	12		0-3	2-4	0	
ducurbita palmata, S. Wats.			3-4	4	1-4	
Cucurbita foetidissima H. B. K.			3-4	4	1-4	
yclanthera explodens Naud			0	$\bar{2}$	0-1	
Cyclanthera pedata Schrad.			0-2	0–2	4	
Echallium elaterium (L.) A. Rich			0-1	0 %	ō	
Echinocystis macrocarpa Greene			0-2		0-2	
Lagenaria leucantha (Duch.) Rusby			3-4	4	3-4	
Luffa acutangula (L.) Roxb.			0 -	ō	0 =	
Luffa aegyptiaca Mill.			Ŏ	Ŏ	0	
Crichosanthes anguina L.	20		0-1	0-4	0-4	

a 100 plants or more.

(wild gourd), C. palmata S. Wats. (coyote melon), and Echinocystis macro-carpa Greene (Chilicothe mock cucumber). We have found powdery mildew on plants of Cucurbita foetidissima and C. palmata growing in the wild. This indicates that the susceptible native flora may be a source of the fungus for infecting cultivated plants.

No species closely related to *Cucumis melo* was found with resistance of a higher order than that found in *C. melo* itself. Those genera and species having virtual immunity from powdery mildew are probably all too distantly related to be of value in attempted hybridization with *C. melo*. The failure to find resistant individuals in a species is no proof of complete susceptibility in that species. Had greater numbers of plants been tested, some resistant individuals might be expected because of the heterozygosity of many of these species. The finding of resistance, however, is definite, since the possibility of escape from infection under the conditions imposed is extremely remote.

United States Horticultural Field Station, La Jolla, California.

b The mildew reaction often is the range found between plants, indicating, in the case 0-4, that some plants were completely immune while others were highly susceptible. Thus in some species there is considerable heterozygosity insofar as resistance to powdery mildew is concerned.

TECHNIQUE FOR HASTENING FOLIAGE SYMPTOMS OF PSOROSIS OF CITRUS¹

JAMES M. WALLACE 2

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INTRODUCTION

The symptoms of the psorosis virus diseases of citrus have been described recently by Fawcett and Bitancourt (4). As pointed out by these authors, the young-leaf symptoms are common to several related viruses, or forms of the same virus, affecting citrus. Although the young-leaf symptoms of psorosis are sometimes very conspicuous on diseased trees in the field, they are so variable and so difficult to detect under some conditions that their absence does not necessarily prove that the tree is free of the virus. Furthermore, the scaling or bark-lesion symptom (4) of psorosis commonly does not develop on orchard trees until the trees are from 12 to 16 years old, or older, even though the trees have been systemically infected throughout this time, having been propagated from diseased buds.

Fawcett (1, 2, 3) demonstrated that the buds and bark of psorosis-affected trees contain virus, and that propagation from diseased trees is the only important means of increase and dissemination of psorosis throughout the citrus-producing regions of California.

Fawcett and Cochran (5) were able to infect healthy trees by grafting to them small pieces of bark from diseased trees. Their method consisted of removing a small disk of bark from the tree to be inoculated and replacing it with a disk of equal size from a diseased tree. The bark disks were removed with a cork-borer, and the completed graft was wrapped with grafting cloth. These writers showed further that scaling could be induced on inoculated trees in less than 5 months if bark patches were transplanted directly from lesion areas to healthy trees. On the other hand, if the patches were taken from normal bark 3 to 6 inches away from the lesions, no bark symptoms had developed on inoculated trees within 3 years. This suggested that a long time would elapse before bark symptoms would develop on such experimentally infected trees, as is the case when trees are propagated from normal-appearing buds of diseased trees.

The transmission studies of Fawcett (1, 2, 3) and of Fawcett and Cochran (5) consisted primarily of inoculations of field and nursery trees. No particular study was made of the time required for symptoms to develop on the young leaves of experimentally infected trees, but Fawcett observed such symptoms in 5 weeks on shoots that developed from diseased buds placed in healthy trees.

In 1942 the writer began some investigations on this group of virus dis-

¹ Paper No. 527, University of California Citrus Experiment Station, Riverside, California.

² Associate Plant Pathologist in the Experiment Station, University of California.

eases of citrus. One of the first studies was for the development of a standardized inoculation technique for use with these viruses, and, particularly, for decreasing the time required for leaf symptoms to appear. A useful inoculation technique has evolved that commonly induces leaf symptoms on young trees within 4 weeks after inoculation, and occasionally in as short a time as 15 days.

INOCULATION METHODS

In order to make possible the use of large numbers of plants in greenhouse studies, and to shorten the time required to grow the experimental plants, the present study dealt chiefly with seedling orange trees having a basal trunk diameter of approximately 5 to 8 mm. By seeding in beds under lath and later transplanting individual seedlings to 6-inch pots, it was pos-



Fig. 1. Bark-patch inoculations of seedling orange trees. A. Stock tree prepared for bark graft, showing peeled bark strip, with leaf attached. B. Bark patch (inoculum) in place on stock tree. C. Completed bark graft held in place by rubber tape.

sible in 5 or 6 months to grow trees suitable for inoculation by means of bark grafts. For most of these studies, Homosassa sweet-orange seedlings were used.

The small trunk diameters of the seedlings made it necessary to use rectangular pieces of bark instead of round disks for graft-inoculation of the trees. A location on the basal portion of the seedling trunk, free of thorns or leaf scars, is selected, and the bark is stripped downward after making one horizontal and two longitudinal cuts through the bark with a razor blade. When possible, the point selected for the bark graft is immediately below and in line with an attached leaf (Fig. 1). The horizontal cut is made in the bark above and close to the leaf petiole. The longitudinal cuts extend downward for about an inch, and the distance between these cuts is determined by the circumference of the tree and the size of the bark patch to be inserted. By using the leaf at the top as a "handle," the bark can

usually be separated from the wood by a slight outward pull, the leaf remaining attached at the top of the strip of peeled bark. When the bark does not peel easily or does not loosen easily at the crosscut, a slight pressure at that point with the blade of a knife or scalpel usually loosens it sufficiently so that it can be peeled the remaining distance with the fingers.

The bark to be inserted as inoculum is taken usually from small twigs or limbs of diseased trees, and the area from which it is to be obtained is scraped very lightly with a razor blade to remove the cuticle and epidermal cells. Two horizontal and two longitudinal cuts are then made so as to have a rectangular piece of bark only slightly smaller than the peeled area of the tree This bark is then pried loose at one of the cut ends. to be inoculated. stripped from the wood, and placed in position in the seedling tree, its cambium adjacent to the cambium of the tree. The peeled strip of bark is then folded back in position over the inserted bark patch. If the leaf is still attached to the upper end of the bark to be folded back, it can be held against the trunk with the fingers of one hand while the grafting tape is applied over the completed bark graft with the other. Electrician's splicing compound was found to be excellent for this type of graft. This material is a rubber tape, one side of which is self-adhering. With the inoculation patch in place and the bark strip folded back over it, a small piece of tape is placed over the bark patch and the two ends are pressed together tightly on the side of the tree opposite the bark patch. The inoculated trees are topped a few inches above the bark patch.

Experiments in which the seedling trees were cut back, as in figure 1, in comparison with those in which defoliation or other treatments were employed to speed virus invasion and symptom development, demonstrated that topping gave the best results. Regardless of the growth condition of the trees at time of topping, this treatment stimulated early growth of axillary buds, and when growth was rapid, the leaves developing on the forced shoots sometimes showed symptoms within 15 days after inoculation.

SYMPTOMATOLOGY

The symptoms produced on trees inoculated in the manner just described are variable and seem to be affected by numerous factors. The present paper deals chiefly with the initial symptoms on the young shoots that develop from axillary buds of the topped trees after inoculation. For convenience of description, the symptoms are classified as (a) typical young-leaf symptoms and (b) shock symptoms. One type, or both types, may be induced, the form dependent upon the source of the inoculum.

Typical Young-Leaf Symptoms. Bark-patch inoculations, with bark from infected trees showing neither bark nor twig symptoms, or with normal-appearing bark from trees on which some bark lesions are present, sometimes result only in flecking and stippling of the newly formed leaves (2, 4). Such symptoms are rather typical of those commonly observed on young leaves in the field. Usually, however, the leaves show more chlorotic blotching or

spotting, and frequently some of the affected leaves curve downward. In the greenhouse, the young-leaf symptoms are retained for a longer time than in the field, and, in some cases, as the leaves mature they may show light-colored spots or areas which are not the same as the mature-leaf symptoms described by Fawcett and Bitancourt (4). The young leaves of the succeeding flushes of growth may or may not have symptoms. When such symptoms do reappear, they are similar to those on field trees, and, as on field trees, symptoms occur on only some of the leaves.



FIG. 2. A. Seedling orange tree with shock symptoms following bark-patch inoculation with psorosis virus. Note bending of shoots and loss of leaves on shoots arising in line with bark patch. B. Healthy control tree grafted with virus-free bark patch. Both trees photographed 21 days after grafts were made. All shoot growth developed after trees were topped.

Trees having these symptoms are not noticeably retarded in growth and except for the leaf symptoms are comparable to healthy controls, remaining so unless mature-leaf, bark, or twig symptoms develop on them.

Shock Symptoms. Under certain conditions not yet well understood, orange seedlings infected with psorosis virus by the inoculation methods described herein have shown severe injury on some of the shoots and leaves that develop immediately after the trees are topped. Because such symptoms have not been induced by re-inoculation or by topping of trees previously infected and invaded by the psorosis virus, this reaction is described

as a "shock" stage of the disease. The first indication of this severe type of symptom is that some of the newly developing shoots begin to twist slightly, and as growth continues the shoot becomes bowed or curved. The leaves that have formed also curve downward and drop upon the least disturbance. The tiny growing-point leaves wither and drop, necrotic spots sometimes appear on the leaves and the young stems, and at later stages the shoots die back partly or completely to the main trunk. The dead, twisted shoots dry and darken and frequently remain attached to the tree for a long time.

In figure 2, the two upper shoots of tree A show the bending and loss of outer leaves typical of a tree in the shock stage. The short shoot, fourth from the top, is completely defoliated. Tree B is a control plant which received a virus-free bark patch. As a rule, only those shoots arising in line with the bark patch will show the shock reaction. Commonly, the shoots on the opposite side will appear normal, although at later stages they may have only typical leaf symptoms.

The shock symptoms have been obtained only with the bark-scaling varieties of psorosis, psorosis A and psorosis B, and are not always induced by inoculations with these two varieties of the virus. So far, the results indicate that with certain strains of the psorosis-A virus, the shock stage of symptoms is induced by bark-patch inoculations irrespective of whether the inoculum is from normal-appearing bark or directly from psorosis bark lesions. The severe shock reaction has resulted in numerous instances when the bark patch used as inoculum was from trees that had no bark, twig, or matureleaf symptoms. Thus, the shock reaction, occurring within 2 to 4 weeks on the newly developing shoots of small seedling trees that are cut back when the bark graft (inoculation) is made, does not appear to be influenced by the source of inoculum, that is, whether it is from lesion or non-lesion bark. the other hand, such secondary symptoms as twig and mature-leaf symptoms have developed only on trees of these experiments that were infected from bark from lesion areas of diseased trees. On such trees the twig and matureleaf symptoms have been observed in some instances within 42 days after the inoculation grafts were made.

In six experiments including 105 infected trees on which records were taken 21, 24, and 28 days after inoculation, 82 plants developed symptoms within 21 days or less. Within 28 days, symptoms had appeared on all but three plants. In many tests all inoculated trees became infected but on some occasions the bark patches decayed and no tissue union resulted. Fewer failures of transmission result when the outer bark of the bark patches is scraped slightly to remove surface dirt and organisms. This is particularly desirable when bark from older twigs and limbs is used for inoculum. Even when the bark patches are from young, green twigs, removal of the cuticle and epidermis is of some benefit in that more surface for callus growth and union is provided.

SUMMARY AND CONCLUSIONS

Inoculation of young sweet-orange seedlings having a basal trunk diameter as small as 5 mm. has provided a method of inducing symptoms of the

psorosis diseases of citrus in a short time. Small bark patches from diseased trees, or from trees to be tested, are placed against the cambium beneath strips of bark peeled back for this purpose on the trees to be inoculated. Callus forms rapidly and the virus is transmitted. The small stock trees are topped to within 2 or 3 inches of the bark patch.

Psorosis symptoms, either of a severe shock type or of the more or less typical young-leaf stippling and blotching usually appear on the growth arising from the upper axillary buds after the trees are topped. In some instances, inoculated trees have no symptoms during the first cycle of growth after topping, symptoms appearing at later cycles. This probably results from a delayed movement of the virus into the growing point tissues and its failure to invade the newly forming leaves early enough in their development to induce symptoms. Shock symptoms have developed only on the forced growth of topped trees not systemically invaded by the virus.

Inoculated trees that develop the severe shock stage of symptoms during the first flush of growth commonly have only typical young-leaf symptoms in later flushes and, except for lessened growth during the early shock stage, do not differ from the diseased trees that never showed shock injury. If the bark patches used for inoculum are taken directly from lesion areas, the infected stock trees develop bark and mature-leaf symptoms early, often in less than 2 months, and the trees are permanently retarded in growth.

Inoculation procedure that lessens the time required for symptom development of psorosis-affected orange trees is of value in providing a short-time method for transmission studies. With such technique it is now possible to conduct large-scale fundamental investigations of the psorosis virus diseases, which, because of their peculiarities and their somewhat unpredictable behavior in the field, have yielded slowly to experimental study.

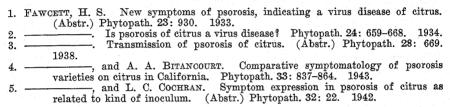
An additional feature of these inoculation methods lies in their use in connection with the registration of virus-free parent trees for propagation of nursery stock. In place of the present practice of periodic inspection of trees proposed as bud-wood parents, and the somewhat delayed and not too satisfactory field-progeny tests from such trees, the inoculation methods described here give promise of providing a short-time test for presence or absence of psorosis virus in trees selected by nurserymen and propagators as bud-wood parents. Although further studies may show that the different, known varieties of the psorosis virus—namely, psorosis A, psorosis B, concave gum, blind pocket, and crinkle leaf, and possibly strains of these varieties—may differ in symptomatology, present indications are that if present in the tissue used for inoculum, any or all of them will induce some type of detectable symptom on the first or second flush of growth of the inoculated trees.

Additional study must be completed of such factors as virus strains, virus concentration, and type of tissue used for graft-inoculation, in relation to symptomatology of psorosis, before it can be determined whether this method can be substituted completely for the present methods of certifying parent

trees. At present this inoculation technique is being used to supplement the regular inspection method of parent-tree certification and has already been of value in eliminating some trees that have been considered for registration. In other instances, this method has provided positive substantiation of the presence of psorosis in certain trees suspected of being diseased, but on which reliable symptoms had not been found in the field. If further investigations show that negative tests by this method can be relied upon as proof that the tree under consideration is free of psorosis virus, it may be possible to use this method of indexing almost exclusively in the registration of disease-free trees for bud-wood parent trees.

University of California, Citrus Experiment Station, Riverside, California,

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THE INVASION OF THE INTERNAL STRUCTURE OF COTTON SEED BY CERTAIN FUSARIA¹

B. A. RUDOLPH² AND G. J. HARRISON³

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Experiments recently reported by the writers of this paper (10) show that cotton seed is an exceedingly insignificant factor in the dissemination of Verticillium wilt in California, if indeed, it is a factor at all. In one phase of the experiments to determine whether the internal structure of the seed may be parasitized by the fungus, cultures were made from 3371 mature cotton bolls produced on plants severely affected with the disease. Of these, the fungus succeeded in reaching the receptacles of only 150 (4.44 per cent) and penetrated as far as the bases of the placental columns of only 2 (0.00059 per cent). It never succeeded in reaching the placentae or funiculi, much less the seeds.

Several species of *Fusarium* were isolated from time to time, not only from the roots, stems, receptacles and bases of placental columns, but also from those tissues which *Verticillium* apparently had been unable to reach, namely, the placentae, funiculi, and seeds.

A number of workers have already reported isolating Fusarium from the interior of cotton seed; Hansford (8) isolated F. moniliforme Sheld. from as much as 25 per cent of the seed with which he worked. He also isolated two species of Fusarium of the section Elegans (7). Similarly, Elliott (2) reported isolating 1, and Crawford (1) 3 unidentified species of Fusarium from within the testa. Not all of these writers expressed opinions as to how the fungus reached the inside of the seed, but in some instances the inference is that it got there by way of the vascular system. Hansford (8) particularly makes it clear that the seed with which he worked was from bolls that showed neither external nor internal evidence of disease, and he was at a loss to explain the presence of the fungus within the seed.

Elliott (2, 3) and Crawford (1) presented seemingly irrefutable evidence that Fusarium vasinfectum Atk. can, and at times does, invade the internal structure of cotton seed and, because of this, they concluded that the disease is seedborne. Such a conclusion is not valid, because of the possibilities that the fungus may (a) die in the seed before it is planted in the spring, thus leaving it as healthy as any other; (b) kill the embryo, thus preventing the production of a plant (Taubenhaus and Ezekiel (11) found this to be true to some extent); or (c) not be able to attack the seedling from its position within the seed, assuming that the fungus is capable of living until planting time in the spring and that it does not kill the embryo. Regardless of whether the embryo be killed, if the fungus can live over in the seed,

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Associate Plant Pathologist in the California Agricultural Experiment Station.
 Agronomist, United States Department of Agriculture.

there is still the possibility that it can establish itself in the soil when suc seed is planted.

Neal (9), Gilbert (6) and Fahmy (5) deny that Fusarium wilt is seed borne and base their contention on the fact that, in their experiments, see taken from diseased plants produced healthy plants when planted the fol lowing spring. They did not question, however, whether some of the seed might have once contained the fungus in a living condition. Ezekiel (4, 11 and Taubenhaus (11), on the other hand, not only isolated Fusarium vasin fectum from seed taken from diseased plants but also secured diseased plant when they planted the seed under controlled conditions.

Elliott (3) and Crawford (1) have shown that the incidence of interna infection in seed is not great, also that the viability of the fungus may be expected to decline sharply three months after the seed is harvested. Thus by planting time, the seed containing the viable fungus may constitute a very small percentage of the total and might very easily be missed when limited numbers of cultures are made.

The sharp disagreement existing in the literature today as to whether Fusarium wilt is seedborne stems largely, on the one hand, from the invalid conclusion by certain workers that the mere presence of the fungus within the seed constitutes proof that the disease is seedborne and from the equally invalid conclusion by certain other workers that it is not seedborne because seed from diseased plants usually yields healthy plants when planted the following spring. Apparently time and incidence factors, overlooked by many workers, are involved, as the works of Taubenhaus (11), Ezekiel (4, 11), and Elliott (3) seem to show. The incidence of infection in seed is lower in some years than in others, and there is a tendency for the fungus to die out rapidly after a time in the seed. Also, a certain amount of the seed is killed by the fungus.

SEED STUDIES IN CALIFORNIA

The technique employed in the experiments which showed that internal infection of cotton seed by Verticillium albo-atrum rarely if ever occurs in California has been described in considerable detail (10). Cultures were made in the approved, conventional manner. The results, however, were considerably different from those anticipated: whereas Verticillium seemingly was unable to reach the placentae, several species of Fusarium, which, with the Verticillium, inhabited the xylem of roots and stems although its presence there was not suspected, frequently reached the placentae. Table 1 shows the percentage of bolls, the placentae of which yielded Fusaria in culture in 1935 and 1936. The bolls were fully mature but unopened and, aside from being more or less stunted, as they are when the plants have Verticillium wilt, they had no disease symptoms whatever, either externally or internally.

Since the only symptoms of the plants from which the bolls were taken were those ordinarily associated with Verticillium wilt, a question arose as

to whether any of the several Fusaria associated with Verticillium in the plants might be an active parasite, capable of attacking cotton seedlings per se and producing definite symptoms of its own. To prove this point, cotton seed produced by cotton plants to all appearances healthy at the U. S. Cotton Field Station at Shafter, California, was planted in sterilized pots and large wooden boxes in the greenhouse at the Deciduous Fruit Field Station at San Jose. The soil was sterilized in a steam pressure cooker at a local cannery at temperatures varying from 250° to 268° F for 9 to 18 hours, the lower the temperature the longer the time allowed for sterilization. The seed was delinted with concentrated sulphuric acid, washed, dried, and then examined under the microscope for the tiny, bristle-like hairs that often persist at the stem end of a seed, even though the testa itself may have been charred almost to destruction by the acid. These bristles, when present, were scraped off with a scalpel and the seed was surface-sterilized by immersion in HgCl₂, 1/1000, for 5 minutes. It was then washed with sterile water and planted.

TABLE 1.—Isolations of Fusaria made from the placentae in bolls of cotton plants known to be affected with Verticillium wilt

Year	Diseased plants	Bolls cultured		centae infected usarium
	No.	No.	No.	Per cent
1935	12	61	5	8.19
1936	53	434	32	7.37

When the young plants were about 6 inches high, the soil of a given lot of pots or boxes was inoculated with homogeneous cultures of one of the several species of Fusarium originally isolated from the internal tissues of cotton seed, a second lot with another species, a third with another, and so on. The inoculations were made as follows: A sterile steel bar 1 inch in diameter was pressed into the moist soil to a depth of 6 inches immediately alongside each of the plants. Then a fresh 10-cc maximal slant culture of the fungus, growing in a standard culture tube $\frac{3}{4}$ inch in diameter and 6 inches long, on 2 per cent Czapeck's solution agar, was scraped into each hole. The hole was then pinched shut. Check plants were similarly treated except that inoculum was not placed in the holes.

Cotton does not grow well in the close confinement of pots or even large boxes. The plants were small, but none developed disease symptoms of any kind. In the steles of occasional plants were faint brownish streaks, but there was no relation between the discolorations observed and the results obtained with cultures.

Cultures were made of tissue taken from the steles of all plants at least 6 inches above soil level, on Czapeck's solution agar slightly acidified with lactic acid to suppress bacterial growth. At least 4 months had elapsed since the soil had been inoculated. The plants were fully mature and had produced bolls. The results obtained in three different years are in table 2.

TABLE 2.—Results of cultures made from the steles of cotton plants grown in steam sterilized soil infested with Fusaria isolated from seed

Year	$\begin{array}{c} \text{Plants grown in} \\ \text{soil infested with} \\ \textit{Fusarium} \end{array}$	Check plants	Plants infected	l with Fusarium
	No.	No.	No.	Per cent
1937	60	29	33 5	55.00 17.44
1938	46	18	19	41.30 0.0
1941	32	 6	7 4	21.87 66.66

It was perfectly clear from the results obtained in 1937 that the so-called healthy seed from which the plants were grown must have harbored the Fusaria internally; the severe preliminary treatment given the seed, consisting of delinting with acid, hand scraping, and disinfecting, precluded all possibility of the fungus being carried on the exterior. Yet both the inoculated and check plants yielded the same species of Fusarium abundantly in culture. Not infrequently plants yielded one or more additional species of Fusarium other than the one used to inoculate the sterile soil of a given lot of pots or boxes. Such species could have come from only one possible source, namely, the so-called healthy seed from which the plants were grown. And internal infections of such seed must necessarily have taken place in the field by way of the vascular system, since the bolls showed no internal or external evidence of disease whatever at the time the seed was harvested. Original infection of the plant takes place through the roots, but no lesions or other symptoms were on the roots at the time cultures were made from the steles. In 1939 the percentage of infection in the checks actually exceeded that in the plants grown in infested soil. Apparently 1937 was not favorable to natural infection in the field, because the check plants raised in 1938 from seed produced in 1937 were not infected.

Additional evidence that the Fusaria inhabit the interior of the seed was obtained. Seed taken exclusively from bolls the receptacles of which had yielded Fusaria in culture was delinted with concentrated sulphuric acid, scraped free of bristles, disinfected and washed, and planted in the field at the Deciduous Fruit Field Station where cotton had never grown before. Checks were provided, but the seed used was so-called healthy seed

TABLE 3.—Results of cultures made from the steles of field-grown cotton raised from seed taken exclusively from bolls the receptacles of which had yielded Fusaria in culture

Material cultured	Plants infect	ted with Fusarium
	No.	Per cent
104 plants raised from seed taken from bolls with receptacles infected with Fusarium	26	25.00
111 plants raised from healthy seed. Check	22	19.81

obtained from the Shafter Station. Both lots of plants were of equal vigor and without disease symptoms of any kind.

When the plants were 5 months old, cultures were made from stele tissue taken at least 6 inches above ground (Table 3).

The percentage of plants infected with Fusaria was higher in the lot raised from seed taken from bolls the receptacles of which had yielded the fungus in culture; but it was also high in plants raised from so-called healthy seed, which indicates that the fungus was present in much of the seed at the time it was planted. A certain amount of infection may have taken place, of course, in the soil which was not sterile and which may have harbored the Fusaria, but both lots of plants were equally exposed.

Internal Infection of the Seed. To make sure that the Fusaria may penetrate the interior of the seed by way of the vascular system, seed from bolls the receptacles of which had yielded Fusaria in culture was saved, acid delinted, scraped free of bristles, surface-sterilized, washed, and cul-

TABLE 4.—Results of cultures of cotton seed from bolls the receptacles of which had yielded Fusaria in culture

Year	Bolls with infected receptacles	Seed in bolls	Seed infe	ected with Fusarium
	 No.	No.	No.	Per cent
1938 1939	10 19	277 537	$\begin{array}{c} 72 \\ 113 \end{array}$	$25.99 \\ 21.04$

tured. Each seed was cut transversely into halves with sterile instruments and planted in Petri plates of slightly acidified Czapeck's solution agar. To maintain the identity of each seed, the halves were planted in proper sequence on either side of a line drawn across the bottom of each plate with a wax pencil, the funicular halves on one side and the micropilar on the other. Not more than 4 seeds (8 halves) were planted to a plate. More than $\frac{1}{5}$ of the seed was infected internally in 1939 and $\frac{1}{4}$ in 1938 (Table 4).

Field Inoculations. Because cotton grows poorly in pots, the greenhouse studies (Table 2) were repeated in the field at both the San Jose and the Shafter stations. Inoculations were made in the spring when the plants were 6 inches high, and checks were provided. The seed used for planting at San Jose was acid delinted, hand scraped, disinfected, and washed; that used at Shafter was treated with Ceresan at the rate of 3 oz. per 32 lb. of seed. Cultures were made from the steles at least 6 inches above ground level in the late fall or early winter when the plants were fully mature and earrying many bolls. The results are in table 5.

At Shafter and at San Jose all plants grew normally in the field and had no disease symptoms of any kind. Again, as in the case of the pot experiments (Table 2), the percentage of infection in the check plants was high, in 2 instances actually higher than that of the plants grown in infested soil. The reason for this is not clear.

Identification of the Fusaria.⁴ The experiments reported here were conducted entirely as a side line in connection with a major project to determine whether Verticillium wilt is seedborne. Time did not permit a detailed study of the many cultures of Fusarium isolated from receptacles, placentae, and seed. Moreover, when it was recognized as the result of inoculation experiments that these Fusaria were entirely innocuous and seemingly natural concomitants of healthy plants and seed, there seemed to be no occasion for more precise studies. Since the Fusaria discussed here presumably inhabit the xylem of cotton plants simply as saprophytes, or at most as very weak parasites, the invaded plants and seed, in a broad sense, can be regarded as being as healthy as those in which these fungi do not occur. This view is not extreme. Somewhat similar, but not entirely analogous,

TABLE 5.—Results of cultures made from field-grown cotton plants produced from healthy seed and grown in soil infested with Fusaria

	Plants grown in soil infested with Fusarium	Check plants	Plants infected v	with Fusarium
	No.	No.	No.	Per cent
San Jose, 1940	136	13	$rac{6}{2}$	4.41 15.38
San Jose, 1941	115	30	51 12	44.34 40.00
Shafter, 1941	483	104	88 22	18.21 21.15

instances can be found in the higher animals, including man, whose lower intestines swarm with saprophytic organisms. Such a situation is general, and the individual is not considered diseased because of their presence.

Among the Fusaria isolated from xylem in all parts of the plants and from the seed, and used in the inoculation experiments, were *Fusarium moniliforme* Sheld., *F. oxysporum* Schl., and *F. scirpi* Wollenw. All three have been reported on cotton. In addition there was another species, the identity of which was never determined. Unfortunately a culture set aside for study became contaminated and died.

With the exception of Fusarium scirpi, none of the species appeared to injure the seed. F. scirpi not infrequently was isolated from immature seed that had broken down with a soft, brownish decay. The bolls themselves were to all appearances healthy. Yet it is evident that this fungus does not always kill the seed, since plants grown from acid-delinted seed in sterile soil often yielded F. scirpi when cultured. It was distinctly less frequently isolated, however, from either plant stems or seed than were the other species.

SUMMARY

Several species of Fusarium appear to be innocuous concomitants of healthy cotton seed. They reach the seed by way of the xylem which they

⁴ Grateful acknowledgment is made to Dr. W. C. Snyder for his invaluable assistance in identifying the Fugaria

seemingly inhabit as saprophytes, since the plants have no disease symptoms whatever. Two have been identified as F. moniliforme Sheld. and F. oxysporum Wollenw. A third, F. scirpi, is capable at times of producing a soft, brown rot of immature seeds. A fourth species remains unidentified. Had time permitted more detailed study, additional species or strains might have been recognized.

University of California Deciduous Fruit Field Station, San Jose, California,

AND

U. S. COTTON FIELD STATION, SHAFTER, CALIFORNIA.

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THE FIELD REACTIONS OF CERTAIN OATS HYBRIDS AND VARIETIES TO STEM RUST¹

M. B. MOORE, H. K. HAYES, AND E. C. STAKMAN (Accepted for publication March 12, 1945)

In the course of a breeding program for the production of disease-resistant varieties of oats, crosses were made in 1930–1933 between Bond and each of the varieties, Rainbow, Iogold, Anthony, "Double Cross" A,² and "Double Cross" B.² Selected lines from these crosses have been consistently resistant to stem rust *Puccinia graminis avenae* Erikss, and Henn. in the field until 1943. In this year, however, a liberal sprinkling of large pustules, in addition to the usual number of small ones, appeared on nearly all of the lines from Bond × Rainbow and Bond × Iogold crosses. These large pustules indicated the presence of races to which these varieties are susceptible. The lines from Bond × Anthony and from Bond × "Double

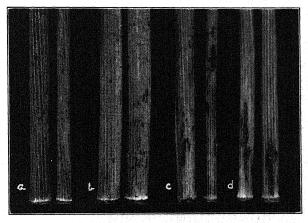


FIG. 1. Stem rust infection types on field grown plants. Semi-resistant type pustules on, a, Bond \times "Double Cross"; b, Bond \times Anthony. Semi-resistant and susceptible type pustules on c, Bond \times Rainbow. Susceptible type pustules on d, Tama.

Cross'' A and B, on the other hand, were resistant or semi-resistant as indicated by the development of the small pustules characteristically produced by races 2 and 5 on lines from all these crosses. On all of 26 lines of Anthony or "Double Cross" parentage there were only small pustules, and the lines were clearly semi-resistant or resistant. Of 25 lines of Rainbow or Iogold parentage, however, only 3 were completely semi-resistant or resistant, while both large and small pustules appeared on 22 lines (Table 1 and Fig. 1). Vicland and Tama likewise had both types of pustules, and in plots in various locations in Minnesota the prevalence was as high as 10 per cent, with the severity in some cases almost as high.

Paper No. 2213 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.
 Lines from (Minota × White Russian) × Black Mesdag.

TABLE 1.—Classification of lines of oats for field reaction to stem rust at University Farm, St. Paul, Minn. in 1943

Cross	No. of lines	No. of lines resistant or semi- resistant	No. of lines with resistance and susceptibility in same plant
Bond × Anthony	10	10	
" × Double Cross A	14	14	
" × Double Cross B	2	2	
" × Rainbow	15	3	12
" × Iogold	10		10

The occurrence of the two types of pustules, often on the same plant, strongly suggested the presence of some race of stem rust other than the usual races 2 and 5. For this reason collections were made from each of the crosses and from Tama and Vicland and the rust was then identified in the greenhouse (Table 2). All of the 9 collections from lines or varieties having Rainbow, Iogold, or Richland parentage (Tama and Vicland from the last) yielded only race 8, while 3 of the 4 collections from the Anthony or "Double Cross" lines yielded only race 2 and one of them yielded 70 per cent of race 2 and 30 per cent of race 8. Such an occasional isolation of race 8 from these crosses, supposedly resistant to it, is to be expected since race 8 regularly causes small resistant type pustules on them.

It has recently been shown by Stakman and Loegering (3) that races 8 and 10, closely similar races, have increased during the last few years to 20.2 per cent of all of the collections made in the United States in 1943. In the same year races 2 and 5 comprised 79.1 per cent of the collections.

Presumably the resistance of the Anthony and "Double Cross" hybrids traces back to their White Russian parentage. Levine and Smith (1) found that White Russian and certain White Russian derivatives were resistant to races 1, 2, 5, 8, 9, and 10 and susceptible to 3, 4, 6, and 7 while Rainbow, Iogold, and Richland were resistant to 1, 2, 3, 5, and 7 and susceptible to

TABLE 2.—Races of stem rust identified from lines and varieties of oats2

Cross or variety	Number of lines from which rust races were identified	Races ^b and number of times identified
		Races 2 & 5 Races 8 & 10
Bond × Anthony	2	2
" × Double Cross A	$ar{1}$	
" × Double Cross B	ī	Î
" × Rainbow	5	
'' × Iogold	2	Š
Tama	회사한 하루 하다는 경험	지, 음식하다 하고 있는 그가 📅 되는 다.
Vicland	발표되었다. 🔓 이 보고 안하다	

<sup>a Inoculum taken from large susceptible type pustules whenever they were present.
b No distinction was made between races 2 and 5 or between races 8 and 10 since they differ only by a 4 or an X infection type on one differential variety.</sup>

4, 6, 8, 9, and 10. Smith (2) obtained evidence that resistance of the White Russian type to races 1, 2, and 5 was allelic to the resistance of Rainbow to 1, 2, 3, 5, and 7. He believed the same factor pair was responsible for reaction to races 8 and 9. As White Russian derivatives proved resistant in 1943 when race 8 was present, this is further indirect evidence regarding the White Russian factor for resistance. Welsh (4) in 1937 reported that lines obtained from a cross of Hajira and Joanette were resistant at low temperatures to races 1, 2, 3, 4, 5, 6, 7, 8, and 10. Nevertheless, resistance of seedlings to races 1, 4, 5, and 6 was influenced by temperature, and there was no surety that the resistance would be adequate under field conditions if high temperatures prevailed. It appears impossible to decide which of the three types of resistance to stem rust is most desirable, although the White Russian type was more satisfactory than the Rainbow-Iogold-Richland type in 1943.

The role of the barberry in permitting hybridization between races of *Puccinia graminis* and the resultant production of races having new pathogenic capabilities is well known. The possibility of virulent races acquiring, by the same means, factors for rapid multiplication, cold resistance, longevity—in short, for success in nature—should not be overlooked. Nearly all of the stem rust-resistant varieties of oats that have been grown extensively in northern United States have been of the White Russian or Rainbow-Iogold-Richland types as concerns reaction to stem rust. For a considerable number of years their resistance has been sufficient under practical field conditions to protect them against appreciable injury from stem rust. The field reactions of Richland, Rainbow and Iogold hybrids in 1943 show the desirability of further efforts to obtain oat varieties resistant to all prevalent races.

MINNESOTA AGRICULTURAL EXPERIMENT STATION, St. Paul, MINNESOTA.

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NIGROSPORA ORYZAE (B. AND BR.) PETCH ON MAIZE¹

J. H. STANDEN²

(Accepted for publication, March 17, 1945)

INTRODUCTION AND REVIEW OF LITERATURE

A rot caused by Coniosporium gecevi B. and K. in ears of maize in Bulgaria was described in 1912 by Bubak and Kosaroff (3). Durrell (6) in 1925 found that the same fungus, which he referred to Basisporium gallarum Moll., invaded maize kernels and injured the embryo. He observed that infection was more prevalent in early varieties than in late ones, and that the disease was severe following heavy rains in August and September. Savulescu and Rayss (19 through 25) found that Nigrospora infection of maize in Roumania was prevalent in varieties with a high water content in the ears at time of harvest. According to their observations, late varieties were more frequently infected than early varieties. This was an apparent contradiction of Durrell's observations. According to Raleigh (17) Nigrosporainfected seed often produced plants with normal ears if there was no competition in the hill, but in competition with strong, healthy plants, plants from such infected seed seldom produced normal ears.

Reddy (18) reported that infection by Nigrospora occurred after translocation ceased in the plant. He found that Nigrospora-infected seed suffered the most damage at temperatures too low for rapid germination. Seeds of certain inbred lines of maize germinated at lower temperatures than others and suffered less damage from infection.

Seed treatment was most beneficial under conditions unfavorable to germination of the seed. Reddy found that incidence of infection was lower in cobs with a low pH than in those with a high pH.

In an effort to extend and clarify our knowledge of this disease a study was made on the pH of the cob, on the maturity of the host in relation to prevalence of infection, and on the overwintering of the fungus.

DESTRUCTIVENESS, GEOGRAPHICAL DISTRIBUTION, AND PREVALENCE OF THE DISEASE

Infection of maize plants by Nigrospora has been reported so widely that the disease probably exists wherever maize is grown. The fungus, under several different names, has been reported on maize in North America, Asia, Europe, New South Wales, and Java (Table 1). In 1939 the author collected it on an ear of ceremonial corn obtained from the Indian Day School, Taos, New Mexico.

1 Journal paper No. J-1234 of the Iowa Agricultural Experiment Station, Ames, I Journal paper No. 3-1254 of the lowa Agricultural Experiment Station, Towa, Project No. 95. This paper constitutes one section of a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

² The author wishes to express his gratitude to Drs. C. S. Reddy and I. E. Melhus for their guidance in this research, and to the many others who have given valuable

Although the disease is widespread, most of the estimates of losses to the corn crop have been made for the corn belt of the United States. The loss in Iowa for 1923 to 1939 was estimated at 3 per cent (Iowa Plant Disease Survey records of the Botany Department, Iowa State College). In 1923 a loss of 30 per cent was recorded for certain cornfields in the State, while in 1925 the average loss for the State was 8 per cent. From 1924 to 1938 Nigrospora dry rot caused a loss in two or more years of 0.5 per cent or more in Illinois, Wisconsin, Indiana, and Iowa, according to reports published in Plant Disease Reporter Supplements. Losses were more serious in some years than in others. The disease was also reported during this period from

TABLE 1.—Geographical distribution of Nigrospora oryzae on maize and synonyms under which the fungus has been reported

Locality	Fungus	Date
North America		
Ohio	Coniosporium gecevi Bubak	1912 (1)
Iowa	Basisporium gallarum Moll.	1925 (6)
Manitoba, Canada	Basisporium gallarum	1938 (2)
New Mexico	Nigrospora oryzae	1939
Europe		
Italy	Trichosporium maydis Sacc.	1911 (12)
Bulgaria	Coniosporium gecevi Bubak	1912 (3)
Roumania	N. oryzae (B. and Br.) Petch	1930 (20)
North Caucasia	N. oryzae	1933 (11)
Asia	'뭐임기'()	()
Java	Nigrospora javania Zimm.	1918 (16)
Western Siberia	Basisporium gallarum	1935 (14)
Africa		
Gold Coast, Africa	B. gallarum	1926 (4)
Kenya Colony, Africa	N. sphaerica (Sacc.) Mason	1929 (13)
Tanganyika Territory, Africa	N. sphaerica	1931 (28)
Australasia		()
New South Wales	B. gallarum	1931 (15)

Connecticut, Ohio, Kansas, Missouri, Minnesota, and Massachusetts. Hoppe and Holbert (8) isolated *Nigrospora* frequently from damaged kernels from corn belt states, but from only a small percentage of the kernels from southern and western United States. Koehler and Holbert (10) estimated an average annual loss in Illinois of a little over 1 per cent, which may not be too high for the corn belt states as a whole.

Savulescu and Rayss (20) have reported the disease in Roumania, where the fungus caused important losses in dent corn.

The fungus invaded the maize shank, the cob, and the kernels. Durrell (6) found that shanks of infected ears were at times so severely rotted that the ears were knocked to the ground by mechanical pickers. The invasion of the seed, however, probably is responsible for the larger part of the loss caused by this fungus.

Durrell (6) showed that the viability of infected seed was considerably lower than that of nearly disease-free seed. Koehler, Dungan, and Holbert (9) in 1928 found that, on good soil, seed infected with *Nigrospora* yielded 59 bushels as compared with 65 bushels for nearly disease-free seed.

Many of the inbreds and single crosses employed in producing hybrid seed corn were very susceptible to Nigrospora. In 1938 infection was found in 11 of 42 ears of inbred L-317, 9 of 30 ears of Os-426, and 28 of 38 ears of I-205. In the same year corn in a 40-acre crossing block in eastern Iowa was so severely infected as to be unfit for seed.

Seed corn may appear free of disease and still be severely infected with Nigrospora, as indicated by the following experiment. Two rows of kernels were taken from each of 20 ears known to be infected. After removal of kernels that appeared to be damaged, samples were submitted to each of three Federal grain grading agencies, by which they were given a uniform grade of No. 1 sound corn. Kernels were then surface disinfected and dropped on agar slants. Nigrospora was on 334 of 408 kernels, Fusarium

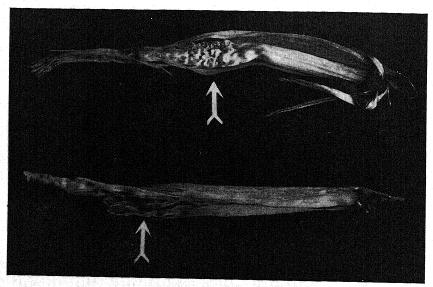


Fig. 1. Arrested axillary shoots of corn infected by Nigrospora. Arrows point to areas severely retted by the fungus.

or other fungi occurred on 58, while 16 appeared to be disease-free. Infected kernels failed to germinate or germinated less vigorously in the agar slants than healthy kernels.

Almost all references to Nigrospora in the literature are concerned with the dry rot of the ear. Ellis's collected infected maize culms in New Jersey, and Durrell (5) reported nodal infection. The author has published a note (26) on the prevalence of this fungus in arrested axillary shoots and poorlydeveloped secondary ears. In some collections, each of 40 or more arrested axillary shoots were infected. Collections with 50 to 80 per cent of the shoots infected were general. Infection was far more common in small, poorlydeveloped secondary ears than in ears that were well developed. Two Nigrospora-infected arrested axillary shoots are shown in figure 1. The Nigro-

² No. 968, North American Fungi, J. B. Ellis.

spora in these tissues, while of little economic importance in itself, probably provided an abundance of field inoculum. Spores from infected axillary shoots overwintering in the field were viable in October of the following year.

OVERWINTERING AND GERMINATION OF NIGROSPORA SPORES

An experiment was arranged to gain some idea of the rôle of the abundant inoculum produced in arrested axillary shoots and poorly-developed secondary ears in the initiation of infection the following season. In the fall of 1938 one lot of infected maize tissues, including ears and arrested axillary shoots, was buried 2 inches in the soil. A second lot was placed on the surface of the soil and covered with a screen wire frame to prevent dispersal by the wind. At intervals in the following spring spores from the two lots and from stalks standing in the field were tested for germination by the method of Durrell (6). The spores were scattered on a microscope slide

TABLE 2.—Viability of Nigrospora spores from infected maize tissues buried in the soil, stored on the surface of the soil, and left on standing stalks over winter

Source of spores and date on which overwinter-	Percentage germination of spores in the following spring and summer					
ing began	Mar. 30	June 2	Aug. 10	Oet, 3		
Fall, 1938 Buried tissues Tissues on soil surface Standing stalks	0 15–20 50–60	15 50–60	15–20	10		
	Mar. 26	June 5		Oct. 1		
Fall, 1939 Tissues on soil surface Standing stalks	20 70	15–20 60–65	<u></u>	10		

with a little water and the slide placed in a Petri dish with a few green leaves for 12 to 18 hours. Data from these tests and from tests made on material stored for overwintering in the fall of 1939 are in table 2. The spores from infected tissues overwintering on standing stalks germinated much better than spores from tissues on the surface of the soil, while spores from buried tissues failed to germinate. The experiments with buried tissues were not repeated in 1939–1940. By early June most of the standing stalks with arrested axillary shoots had been plowed down or the stalks had been stripped by the wind. Ten per cent of the spores from tissues on the surface of the soil germinated long after infection had occurred in the field in each of the two years. Spores from material placed on the soil surface in 1938 were viable on Oct. 3, 1939, while similar material stored in the fall of 1939 was still viable on Oct. 1, 1940.

A few spores in tissues stored in the laboratory were viable after five years: spores from a piece of infected cob stored in the laboratory in 1934 were placed in a germinator in 1939, and of 500 or more spores, 2 germinated.

Many of the spores from the tissues that were buried in the ground over

winter were broken in a circumcissal manner which gave them a pill-box appearance. The fracture was perfectly smooth and always in the same plane, namely, a little to one side and parallel to the equatorial plane of the oblate spheroidal spores. Durrell (6) noted a line of fracture extending half way around spores that he had treated with nitric acid.

SLIGHT NIGROSPORA INFECTIONS IN EARS OF MAIZE

Cobs so slightly infected with Nigrospora as to escape detection on microscopic examination were of frequent occurrence. Such infections were detected by soaking the ears in water about 12 hours, incubating at 100 per cent humidity at room temperature for 4 days, and slowly drying for about a week. Shorter periods of incubation did not allow all of the slight infections to become evident. Examination without the drying period likewise did not permit the detection of all of the slight infections. Using this technique, well-matured maize ears were tested from eight Iowa localities in 1937 and 1938. Of 365 ears subjected to microscopic inspection without the treatment, 10 were found infected. Of 365 ears subjected to the soaking, incubation, and drying treatment, infection was detected in 66. This would indicate the possibility that slight infections in the field in early autumn may become extensive if the corn is allowed to remain in the field under conditions favorable to the fungus. Such a possibility is of especial importance in the relation to seed corn.

RELATION OF pH OF COBS TO INCIDENCE OF INFECTION

Reddy (18) observed that corn inbreds in which the pH of the cobs was high were frequently infected with Nigrospora, while infection was less common in inbreds in which the cob pH was low. He examined 410 ears in 1932 in which the pH ranged from 4.40 to 4.59 and found one infected ear. Infection was found in 64 of 248 ears in which the pH ranged from 5.40 to 5.79. In experiments with the effect of pH on growth of the fungus in pure culture no differences in response of Nigrospora were observed over a range of pH exceeding that occurring in the host plant.

In an attempt to explain the relation of cob pH to infection in the field, successive pH readings were made on cobs of corn inbreds with high or low incidence of Nigrospora during 1937 and 1938. The data on these tests are recorded graphically in figure 2. In the early part of the season pH determinations were on the entire immature ears. Kernels in the milk stage were scraped from the cobs before testing. Later tests were on the cobs with kernels removed. Although the pH was uniform for all inbreds early in the season, there was a drop in cob pH of inbreds with a low incidence of infection as the ears became mature. In 1938 three of six cobs in the low incidence groups harvested October 3, had a pH of 5.2, 5.5, and 5.6, respectively. All three ears were poorly matured, and notations before testing were that the pH should be high. The evidence obtained in these experiments indicated that an ear that matured well had a cob with low pH. This fact,

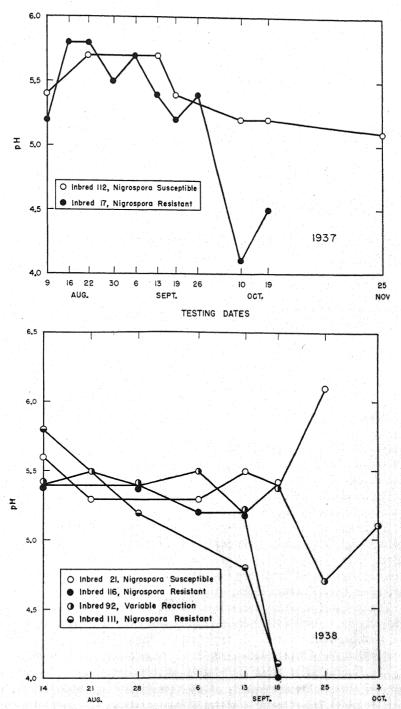


Fig. 2. Seasonal variation in cob pH of Nigrospora-resistant and Nigrospora-susceptible inbreds. (Each point is average pH reading of 3 ears.)

coupled with Reddy's observations and with earlier work by Standen (27), indicates that incidence of infection was probably dependent on maturity.

Inbreds 924 and 21 presented contrasting examples of the relation of maturity to infection. Inbred 21 died early in the field in 1937 and 1938. Inbred 92 was late in maturing in both years. In 1937 three frosts had occurred by the middle of September, and inbred 92 was killed while still immature. Infection was found in 14 of 47 ears in that year. In 1938 the first frost occurred late in the fall at Ames, and only 2 ears of 104 harvested from inbred 92 were infected. Inbred 21 had Nigrospora infection in 15 of 59 ears in 1938. This would indicate that, under certain conditions, inbreds dying prematurely and inbreds that are still immature at time of frost may be attacked by Nigrospora.

THE RELATION OF MATURITY AND DEATH OF THE HOST TO INCIDENCE OF INFECTION

Infection was abundant in arrested shoots and poorly-developed secondary ears. In 1938, infection was observed in 50 of 100 ears harvested from

TABLE 3 .- Relation of maturity of maize plants on September 11, and incidence of Nigrospora infection in the ears on November 19, 1938

Maturity of plants	No. single	No. ears	Ears infected			
	crosses	examined	No.	Per cent		
Well matured	101	4923	243	4.9		
Prematurely dying	69	2717	731	19.7		
Immature	66	3010	206	6.8		

stalks so severely lodged that the ears rested on the ground. In the same field 13 of 603 ears harvested from standing stalks were infected. If the difference in moisture relationship did not entirely account for the difference in infection, this might indicate a relationship between poor maturity⁵ and high incidence of infection. Experiments were arranged to obtain more evidence on this relationship.

A series of 236 single crosses was planted in 1938 to provide material with a wide range of maturity. On September 11 the plants were examined and on the basis of apparent maturity classified into three groups, as prematurely dying or dead, immature, and well matured. The ears were harvested November 19 and examined for infection (Table 3).

The greatest amount of infection was in the group classified as prematurely dying, in which 19.7 per cent of the ears were infected; but the infection (6.8 per cent) in ears of the immature single crosses was significantly greater than the 4.9 per cent in ears from the well-matured crosses.

The fungus grew more abundantly on poorly-matured than on wellmatured ears in the laboratory. Ears were soaked in water for 12 hours

The inbred lines of maize designated by number only are from a series carried by C. S. Reddy of the Iowa Agricultural Experiment Station.

The word "immature" designates living tissues that have not reached maturity.

Poorly matured tissues are those that have died without becoming matured.

TABLE 4.—Inoculation of maize ears with Nigrospora

	Checks			Treated ears		
Corn sample and year tested	Not inco		Not inoculated, incubated		Inoculated and incubated	
ing the second state of Ex	xamined	Infected	Examined	Infected	Examined	Infected
1938						
Commercial corn			19	4	42	35
1939						
Commercial corn	35	0	12	0	35	35
Commercial corn	18	0			18	18
Inbreds	29	3	30	12	30	28
Total	82	3	61	16	125	116

and a portion of Nigrospora culture applied to the butt of the ear. The ears were then incubated and dried as for detection of slight infection. Data from four tests which included untreated checks and ears incubated but not inoculated (Table 4) show that a high percentage of ears that were inoculated and incubated became infected. The commercial corn samples were selected, well-matured ears. In none of these infected ears was sporulation detected at a depth in the cob of more than $\frac{3}{4}$ inch. The inbred ears were poorly matured. Sporulation occurred at a depth exceeding $1\frac{1}{2}$ inches in 22 of the 28 infected inbred ears. In the six ears in which infection was not so extensive, other fungi such as Monotospora or Fusarium were established as well as the Nigrospora. The limited growth of Nigrospora may have been due to these other organisms.

The retting of the cob, a characteristic symptom of the disease in the field, was produced in the laboratory. Twelve samples of poorly-matured cob were autoclaved, with sufficient water to keep them moist, in half-pint bottles and inoculated with Nigrospora in pure culture. Each sample was completely retted in two or three weeks. Twelve well-matured samples subjected to the same treatment were not retted. Growth of the fungus was more vigorous on the poorly-matured than on the well-matured cobs. To twelve samples of well-matured cobs was added extract of poorly-matured cob. Growth of the fungus was abundant on these samples, but the cobs were not retted.

In the retting experiment tests were made for pectinase, using the method

TABLE 5.—Relation of condition of arrested axillary shoot tissues on September 11, 17, and 24, to initiation of Nigrospora infection

	Infection by $Nigrospora$							
Condition of tissues	September 11		September 17		September 24			
VADIACOD .	Examined	Infected	Examined	Infected	Examined	Infected		
Green	3	0	. 6	0	23	0		
Partly green, discolored	1 19	4	4	0	g.224.9	49.000		
Soft and discolored		14	17	13	19	18		

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		Che	Treated ears Inoculated and incubated		
Corn sample and year tested	Not inoculated, not incubated				Not inoculated, incubated
F	Examined	Infected	Examined	Infected	Examined Infected
1938					
Commercial corn			19	4	42 35
1939					
Commercial corn	35	0	12	0	35 35
Commercial corn	18	0	*******	******	18 18
Inbreds	29	3	30	12	30 28
Total	82	3	61	16	125 116

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				Infection by	Nigrospor	a	
Condition of tissues	Septen	aber 11	September 17		September 24		
DISSECTION		Examined	Infected	Examined	Infected	Examined	Infected
Green Partly green, discol Soft and discolored	ored	19	0 4 14	6 4 17	0 0 13	23	0 18

of Harter and Weimar (7) in which the enzyme is detected by its ability to soften the tissues of sweet potato. No pectinase was found in six separate tests with extracts of cobs retted in the laboratory, but in each of four tests with naturally retted maize tissues the pectinase was present.

A study of arrested axillary shoots in 1938 furnished evidence on the relation of maturity and death of the host tissues to incidence of infection. Infection was very common in these shoots. Shoots from inbred lines of corn were examined for evidence of infection on September 11, 17, and 24 (Table 5).

In all collections in which infection could not be detected by the presence of spores, portions of the tissue were cultured. In no case was Nigrospora detected in portions of the shoots that were still green and firm. In the collections made September 24, the 19 soft and discolored shoots, of which 18 were infected, were gathered from consecutive plants of an inbred in which the shoots became soft and disintegrated early in the fall, while the 23 green, firm shoots were taken from consecutive plants of two inbreds growing a few feet from the plants bearing infected shoots. All shoots were rather uniformly exposed to infection, but infection occurred only in tissues that were becoming soft and discolored.

Evidence on the relation of premature death of plants in the field to prevalence of ear infection was obtained in the fall of 1940. Several inbreds died prematurely after heavy rains in late summer. One of these, inbred 113, is shown in figure 3 beside a row of inbred 116, the ears of which were well matured. Infection was found in 99 of 123 ears of inbred 113, and in only one of 126 ears of inbred 116. Inbreds 102, 103, and 104 also died prematurely. Infection was found in 14 of 17, 8 of 24, and 5 of 27 ears of these lines.

Because poorly-matured plants were most severely attacked by Nigrospora and because poor maturity is frequently related to the condition of the plant roots, an experiment was arranged to test the condition of the roots as indicated by the force required to pull the plant from the ground. Two groups of inbreds were tested, one group in which none of the ears was infected in 1938, and the other in which from 10 to 50 per cent of the ears were infected. Consecutive 3-plant hills of each inbred were pulled by means of a lever and spring scale, and the required force recorded in pounds. In 1938 and 1939 the average force required to pull inbreds in which the incidence of infection was low was almost twice that required for inbreds severely attacked by Nigrospora (Tables 6 and 7).

The force necessary to pull a corn plant has a limited bearing on the condition of the ear or of the plant as a whole. The roots may be in good condition, but naturally shallow and therefore relatively easy to pull. This was true of the three inbreds, 90, 74, and 107, in 1938. On the other hand the roots may be in good condition but the stalk severely rotted. This was true in 1939 of inbred L-317, the ears of which were poorly matured. The difference in average pulling force required for the two groups of inbreds, how-

ever, was sufficient to indicate strongly a relationship between root condition and incidence of infection by *Nigrospora*. No attempts were made during the investigation to determine the cause underlying the weakening of the



Fig. 3. Condition of two inbreds, the one with severe Nigrospora infection in ears, the other with little Nigrospora infection following heavy fall rains in 1940. Left, Inbred 116; Nigrospora infection in 1 of 126 ears. Right, Inbred 113; Nigrospora infection in 99 of 123 ears.

maize roots. It was considered possible that inbreds might have a different response, insofar as root condition was concerned, to different seasons and

TABLE 6.—The force required to pull maize plants with high or low incidence of Nigrospora infection in ears, November 20, 1938

High in	${f ncidence\ of\ } Nigros$	pora	Low incidence of Nigrospora				
Inbred line No.	Force in lb. to	pull 1 hill	Inbred	Force in lb. to pull 1 hill			
	Rangea	Av.	line No.	Rangea	Av.		
99	10-164	95.4	105	146-260	221.8		
L-317	52-144	101.2	115	88-270	180.0		
102	52- 96	70.8	116	68-270	164.2		
2	5-128	73.1	91	80-164	113.0		
113	7- 60	32.0	52	84-236	158.4		
L-287	100-260	147.0	90	36-108	72.8		
80	14-164	95.4	30	64-232	151.6		
21	12- 96	55.0	101	88-192	138.4		
81	28- 92	56.4	74	36-184	98.0		
85	10- 80	34.9	107	20-128	87.6		
	Average of all			Average of all			
	hills	76.1		hills	138.4		

^{*} Based on 10 individual hills.

soils. In the light of the evidence presented on the relation of incidence of infection to poor maturity and premature death of the host, the observations of other investigators are of interest.

Hoppe and Holbert (8) observed that *Nigrospora* was important as an ear rot fungus in those years when the maturity of the corn crop was affected by early frost. Koehler and Holbert (10) in describing the symptoms of Nigrospora dry rot, said that severely-infected ears frequently were chaffy. The evidence presented in the present paper indicated that chaffy ears were attacked because of their poor maturity rather than that the chaffiness was caused by *Nigrospora*.

TABLE 7.—The force required to pull maize plants with high or low incidence of Nigrospora infection in ears, October 20, 1939

High in	icidence of Nigrosp	ncidence of Nigrospora				
Inbred	Force in lb. to p	ull 1 hill	Inbred	Force in lb. to pull 1 hill		
line No.	Ranges	Av.	line No.	Ranges	Av.	
La-317	30-200	106.0	115	200-365	285.5	
102	22-126	67.6	116	155-500	336.5	
113	58- 98	77.6	91	102-210	163.7	
2	62-130	82.8	52	50-360	163.5	
L-289	65-260	178.9	90	65-138	107.7	
80	30-102	61.4	30	130-340	252.3	
81	4-178	68.3	101	22-238	148.8	
85	14-238	107.8	74	150-350	210.0	
L-289B	115-170	143.0	107	65-210	101.5	
I-205	50-240	132.0	14	158-270	212.5	
	Average of all			Average of all		
	hills	103.5		hills	191.6	

a Based on 10 individual hills.

According to Ryker⁶ Nigrospora "is not responsible for any disease in rice, although a constant companion of the plant," and he has "never found any indication of its being parasitic. It occurs on almost all growing rice and if for any reason tissue is injured, it develops abundantly in that area. For this reason, it is very bothersome in that in plating of diseased tissue it masks the causal organism."

SUMMARY AND CONCLUSIONS

Poorly-matured maize tissues were more susceptible to infection by Nigrospora than well-matured tissues. Single crosses, separated on the basis of visual inspection into poorly-matured and well-matured groups, had a far greater incidence of infection in the poorly-matured than the well-matured group. Such factors as lodging, stalk rot, root rot, and early frosts, all contributed to increased incidence of Nigrospora infection in ears of maize. Secondary ears and arrested axillary shoots, because of their poor maturity, had a high incidence of infection.

The relation between the pH of the cob and incidence of infection, previously reported by Reddy (18), depended on the lowering of the cob pH as the ear matured. In cobs that did not mature well, or that were not mature at the time of frost, the pH remained high. Late-maturing inbreds were heavily infected in years of early frost, and free of infection when frost was delayed. Green tissues did not become infected.

Infection had been initiated in many maize ears in which Nigrospora failed to spread. The growth of the fungus following infection appeared to be conditioned by moisture, temperature, and the maturity of the cob. The retting of cob tissues, characteristic of the disease in the field, was accomplished in the laboratory, but only by use of poorly-matured cobs. Chaffiness of the ear has been described as a characteristic of the disease in the field. It is probable that chaffy ears are infected because they are poorly matured.

Arrested axillary shoots and poorly-developed secondary ears had a high incidence of Nigrospora infection. It is probable that these tissues constitute a principal source of inoculum for the following season.

IOWA AGRICULTURAL EXPERIMENT STATION, AMES, IOWA.

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ANTHRACNOSE OF GARDEN PEA

S. H. Oul AND J. C. WALKER (Accepted for publication March 17, 1945)

INTRODUCTION

Anthracnose (Colletotrichum pisi Pat.) of pea (Pisum sativum L.) was first collected in Ecuador in 1891 (11). Since then it has been noted in Wisconsin (7, 8, 17), Connecticut (4), Louisiana (12), Canada (2, 5), Japan (6), and Russia (1). The surveys of pea diseases in Wisconsin made by Jones and Linford (7) and Walker and Hare (17) revealed that the disease was very prevalent in both 1924 and 1942.

The disease as it appears in the field has been described by Jones and Linford (7) and illustrated by Walker and Hare (17). Brown lesions occur on leaves, stems, and pods. It was pointed out by Walker and Hare (17) that the symptoms are not readily distinguished in their early stages from those of Ascochyta spp. and that the two diseases often occur on the same plant. Ordinarily the development of setae and sclerotia of Colletotrichum pisi in the lesions has been taken as the basis of distinction of anthracnose from Ascochyta blights. It will be shown later in this paper, however, that Ascochyta spp. and C. pisi occur very commonly together in the same lesion and that on stems, particularly, the latter does not infect independently. It should be pointed out, therefore, that the symptoms which have been ascribed ordinarily to anthracnose are, in fact, those of the combined effects of one or another species of Ascochyta and C. pisi.

The present paper is concerned with isolation and inoculation experiments, the interaction of the causal fungus with $Mycosphaerella\ pinodes$ (Berk. & Blox.) Stone, the Ascochyta stage of which is most commonly associated with $Colletotrichum\ pisi$ in Wisconsin, and the transmission of the latter organism with pea seed.

ISOLATION EXPERIMENTS

Colletotrichum pisi grows readily on common artificial media. Cultures were obtained usually by placing fragments of diseased tissue in 1–1000 mercuric chloride for one minute and plating on potato-dextrose agar. In 1942, many of the resulting colonies contained both C. pisi and Ascochyta spp. Beginning early in the season of 1943, isolations were made at intervals of 7 to 10 days from diseased plants collected in 5 localities in central Wisconsin. Fifty or more lesions were used from each collection. At the time of the first isolations no acervuli or sclerotia were present on the brown lesions. About 75 per cent of the resulting colonies contained Ascochyta spp. as well as C. pisi. Ten days later lesions had appeared on the stems, and setae of the anthracnose organism were present. Young stem lesions bearing

¹ Acknowledgment is made to the China Foundation for the Promotion of Education and Culture for its encouragement to and financial support of the senior author during the period of his graduate studies at the University of Wisconsin.

setae, selected under a binocular, were removed from the stem and plated. Of about 300 colonies, nearly 100 per cent contained both $C.\ pisi$ and $Ascochyta\ spp$. In later isolation experiments, when sclerotia had appeared on the stem lesions, the percentage of colonies containing $Ascoschyta\ spp$. gradually decreased. Thus, it became evident that $C.\ pisi$ was commonly associated with Ascochyta in the field.

INOCULATIONS

Jones and Vaughan (8) obtained positive results on leaves and stems inoculated in the field. Antonova (1) stated that infection of pea seedlings was secured with difficulty and only in the presence of wounds.

The organism, as isolated from naturally infected plants, produced no spores on potato-dextrose agar, but it produced abundant, atypical, cylindrical conidia in a number of liquid media. Conidia produced in modified Duggar's solution² were usually used for inoculation. A strain which produced normal falcate conidia on potato-dextrose agar was later isolated from a sector of the original mycelial culture. This strain also produced cylindrical conidia in liquid media. The pathogenicities of the mycelial and conidial types were compared and found to be similar.

In this study, a large number of inoculations was made in the greenhouse, usually on Perfection or Prince of Wales varieties. At least 3 pots, each containing 10 to 12 pea plants, were used for each treatment. They were inoculated when the fifth or sixth leaf had expanded and the plants were 8 to 10 inches high. Beyond this stage the first or second leaf tended to show signs of senescence, and it was difficult, therefore, to determine whether infection had occurred on living or dead tissue. The pots were placed in a glass moist chamber for 12 hours before and 24 hours after inoculation. Spore suspensions were applied by means of an atomizer. The moisture in the chamber was controlled by an atomizer which was so adjusted that nearly 100 per cent relative humidity was kept without an excess of free water accumulating on the plant. The temperature in the chamber varied from 20° to 23° C.

The results of a number of similar inoculations varied somewhat, but as a rule an average of 3 to 4 leaflets of each plant, usually the lower ones, became infected. The lesions appeared as small, depressed, water-soaked areas within 2 days after inoculation. Unlike the field symptoms, no brown color was evident until the leaflets were dead. Reisolation from these infected leaflets usually yielded the organism.

When inoculation and incubation were carried out at 16°, 20°, 24°, and 28° C., the number of infected lower leaflets increased with temperature. This thermal response was undoubtedly due in part to the influence of temperature upon the growth of *Colletotrichum pisi*. It was also evident, how-

² Composition of the solution: M/2 dextrose, 500 ml.; M/2 KNO₃, 200 ml.; M/4 KH₂PO₄, 200 ml.; M/10 MgSO₄·7H₂O, 100 ml.; M/100 Fe-tartrate, 1 ml.; A-Z solution, 1 ml. The A-Z solution consisted of: LiCl, 0.5 g.; CuSO₄·5H₂O, 1.0 g.; ZnSO₄, 1.0 g.; H₂BO₂, 11.0 g.; Al₂(SO₄)₃, 1.0 g.; SnCI₂·2H₂O, 0.5 g.; MnCl₂·4H₂O, 7.0 g.; NiSO₄·6H₂O, 1.0 g.; Co(NO₃)₂·6H₂O, 1.0 g.; TiO₂, 1.0 g.; KI, 0.5 g.; KBr, 0.5 g.; H₂O, 18 liters.

ever, that at 24° and 28° C. the lower leaves of pea plants became senescent more rapidly, and this was probably an equally important factor in enhancing disease development.

In no case in these and many other experiments were any stem lesions noted up to one month after inoculation. Microscopic examination of inoculated stems and leaflets without lesions showed numerous germinated conidia and many appressoria. In a few cases the inoculated plants were kept in the moist chamber for a longer period. Small colonies of the organism developed on the surface of the stem, but no lesions resulted. The control plants showed no disease except an occasional wilting leaflet which yielded no fungus when plated.

Apparently healthy pods were picked from the field at about the canning stage, washed in sterile water, and inoculated. The pods were kept in moist chambers in an incubator at 20° C. Water-soaked, depressed lesions were evident in 5 or 6 days. The lesions reached 4 to 5 mm. in diameter in about 10 days and usually small, superficial sclerotia appeared at the center. The control showed no disease development. Four lots of pods of 4 varieties were tested with similar results. Examination of a large number of these pods showed that the fungus seldom penetrated through the inner pod wall, and no infection of the seed underneath was macroscopically evident after 3 weeks. Seeds from pods of the same stage were removed aseptically into a small sterilized moist chamber and were inoculated with conidia. Lesions were evident in 3 days at 20° C. Acervuli of the sporulating strain and sclerotia of the mycelial strain began to form within a week.

As previous inoculations failed to induce lesions on the stem, several variations in method of inoculation were made. Plants were left in the moist chamber for 48, 60, and 72 hours instead of the usual 24 hours. They were covered with glass jars to maintain a high relative humidity around them for 7 to 10 days after the inoculation period. The stem was wrapped in a moist cloth following inoculation and the cloth was kept moist by frequent spraying. None of these methods produced stem lesions. When needle wounds on stems were made just before or immediately after the spore suspension was applied, straw-colored lesions developed around the wounds, and, if the stem was kept continuously wet, the lesions occasionally developed up to 6 mm. in length. The fungus was successfully isolated from the diseased tissue. Thus it was shown that the organism developed rather feebly but only as a wound parasite on the stem.

For comparison, parallel inoculations with pycnidiospores of Mycosphaerella pinodes were made. Numerous brown lesions appeared both on stems and leaves. Except for the absence of sclerotia and acervuli, these lesions were comparable or identical to those observed in the field from which Colletotrichum pisi was isolated. The lesions were usually so numerous that they nearly covered the stem and leaf surfaces when the concentration of spores in the suspension was about the same as that used with C. pisi. The inoculated plants usually were killed in 10 or 14 days, although the spore concentration in the inoculum was considerably reduced.

PENETRATION

Penetration of the host by Colletotrichum pisi has been described and illustrated by Jones and Vaughan (8). In view of the irregularity of infection secured in inoculation experiments, the extent of penetration in leaves and stems was studied. At different intervals after inoculation thin layers of tissue were removed, cleared with lactophenol, and stained with cotton blue. A majority of the spores had germinated, and appressoria were abundant at 24 hours. The percentage of infection pegs from appressoria which had pierced the cuticle of the leaf tissue was very low. Moreover, penetration was never observed in stem tissues, although numerous appressoria formed on the surface of stems. This is in accord with the fact that infection of unwounded pea stems was never attained in the inoculation experiments.

INTERACTION OF MYCOSPHAERELLA PINODES AND COLLETOTRICHUM PISI

In view of the fact that anthracnose lesions were found commonly on stems in nature, it was postulated that they resulted when the organism followed a primary invader. Since M. pinodes was isolated most commonly along with C. pisi from such lesions, the interaction of these 2 fungi in infection was studied in the greenhouse. Pycnidiospores of M. pinodes and conidia of C. pisi were suspended together in water and the suspension applied with a brush to stems of pea plants in a moist chamber. Brown lesions typical of M. pinodes resulted. From these brown lesions, however, C. pisi was also reisolated readily. The same results were secured when the spores of M. pinodes were applied first and those of C. pisi 3 days later. Thus it was demonstrated that C. pisi may establish itself readily in pea stems when it infects at the same time as M. pinodes or following it. Under the conditions of these experiments, the rate of disease development was very rapid. The symptoms were more typical of M. pinodes alone, and sclerotia and setae of C. pisi did not develop, as is the case in the field. This difference is believed to be accounted for by the rapidity with which M. pinodes killed plants.

SEED TRANSMISSION

Since seed transmission of Colletotrichum pisi had not been previously demonstrated several hundred seeds from each of several commercial lots were plated on potato-dextrose agar. No C. pisi was found. Twelve hundred seeds from badly diseased pods were removed carefully and plated; from 5 of these, colonies of C. pisi developed. About 200 artificially inoculated seeds were plated at 6 and 10 months after inoculation. All of them yielded C. pisi. This showed that the organism penetrated the seed only occasionally in nature, but once it infected the seed it might live for a considerable period, perhaps a year or more, without losing its vitality. It is also possible that sclerotia are carried with the seed as contaminants.

DISCUSSION

In Wisconsin the prevalence of pea anthracnose in the field during certain

seasons has often given the impression that the disease is a serious one. The isolation and inoculation experiments reported in this study show, however, that although *Colletotrichum pisi* may occur as an independent pathogen on leaves and pods, it is impotent on stems. Its common association with *Ascochyta spp*. on stems indicates that one or another of these fungi commonly serves as the primary aggressor in lesions which are commonly interpreted as "anthracnose lesions" because sclerotia and setae of *C. pisi* are more conspicuous than the fruiting bodies of *Ascochyta*. This is particularly true with stem lesions which can not, apparently, be initiated by *C. pisi*.

In its rôle as a secondary parasite C. pisi is not unlike many other anthracnose organisms. Colletotrichum gloeosporioides Penz. commonly occurs on citrus without becoming an active parasite (3, 10, 15). C. falcatum Went establishes itself only after wounds (19). The cotton anthracnose organism (Glomerella gossypii (South.) Edg.) produces more conspicuous, more numerous, and more typical lesions when associated with the angular-leafspot organism (Phytomonas malvacearum (EFS) Bergey et al.) (18). Colletotrichum circinans (Berk.) Voglino develops on senescent lower leaves of onion but not on vigorously growing leaves (16). C. coffeanum Noack is found on living as well as die-back twigs of coffee, but the primary predisposing factor for the die-back is premature leaf fall due to Hemileia vastatrix B. et Br. (9). Shear and Wood (14) noted in their investigation that leaves and fruits of many plants collected in the field or in the greenhouse developed anthracnose (Glomerella cingulata (Atk.) S. & S.) when placed in a moist chamber. Roberts (13) isolated G. cingulata commonly from apple stem cankers caused by other pathogens.

It would appear from these studies that anthracnose is not so destructive a disease of pea as it may commonly appear upon casual observation. In epidemics of the disease, stem lesions are the most striking evidence of severe damage. In view of the fact that the organism appears to be incapable of acting as an independent parasite on stems, at least without the aid of wounds, and in view of the fact that Ascochyta spp. were isolated regularly from lesions in which Colletotrichum pisi was present, its importance has undoubtedly been overestimated. This overestimation is the more likely because, in the case of Mycosphaerella pinodes particularly as a forerunner, C. pisi fruits and produces sclerotia readily on lesions, while the former produces pycnidia relatively sparsely. It is only upon plating lesions on agar that the presence of the 2 organisms is revealed with certainty. It is also quite possible that other foliage pathogens of pea may act as predisposing agents for the anthracnose organism.

SUMMARY

Colletotrichum pisi has been interpreted occasionally as a serious pathogen of pea in Wisconsin.

Extensive isolation experiments showed that in a majority of cases Ascochyta spp. were isolated along with C. pisi from so-called typical anthracnose lesions.

In greenhouse inoculation experiments no infection of stems was secured except for feeble development around needle wounds, while infection of leaves was confined to those that were mature or senescent. Detached pods were infected when inoculated in a moist chamber.

When stems were inoculated with a mixture of spores of Mycosphaerella pinodes and C. pisi, or when those of the latter were applied several days after inoculation with the former, lesions resulted which were similar to young "anthracnose" lesions secured in the field.

Studies of C. pisi on leaves and stems showed that although conidia germinated readily and appressoria were formed, only a small percentage of the latter produced penetrating hyphae on leaves, while no penetration was observed on stems.

It is concluded that, in the main, C. pisi acts as a secondary pathogen in the field, particularly insofar as the conspicuous stem lesions are concerned.

Five out of 1200 seeds from naturally infected pods yielded C. pisi, showing that the latter may be seed-borne.

UNIVERSITY OF WISCONSIN,

MADISON, WISCONSIN.

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PHYTOPATHOLOGICAL NOTES

Physiologic Specialization in Helminthosporium gramineum Rabh.—Physiologic specialization in the fungus that causes barley stripe has been reported by several workers, but generally the specialization has not been clear-cut. Atlas has been reported resistant to stripe in Wisconsin,¹ although it is susceptible in California.² Recently a stripe culture pathogenic to Atlas was isolated from a naturally infected F₁ plant in the greenhouse at Madison. This culture, designated GHA2, was tested on Oderbrucker (C.I. 4666) and Colsess IV (C.I. 5979), and on Atlas (C.I. 4118) from three sources.

TABLE 1.—Reaction of barley varietics when inoculated with different cultures of Helminthosporium gramineum Rabh., Madison, Wisconsin, 1944

	Stripe reaction ^b of barley varieties								
Culture	Trial ^a number	Wisconsin Atlas	California Atlas	C.I. 4118 Atlas	Oder- brucker Wis. Ped. 5-1	Colsess IV			
		Per cent	Per cent	Per cent	Per cent	Per cent			
C-1	1 2 3 4 Ave.	0 9 3 0 3	0 0 0 0	0 0 	82 91 85 86	90 91 80 87			
GHA2	1 2 3 4 Ave.	50 77 89 72 72	7 71 92 64 59	85 98 92	0 0 0 3 1	68 96 77 80			
45–2	2 4 Ave.	5 0 3	0 0 0	0 0 0	100 93 97	100 100 100			

² Trials were started on the following dates: 1 on Mar. 10, 1944, 2 on Oct. 13, 1944, 3 on Nov. 21, 1944, and 4 on Dec. 8, 1944.

b The reaction in each trial is the average of 2 replications.

The Wisconsin Atlas had been carried in the breeding program for a number of years, the California Atlas was obtained from C. A. Suneson at Davis, and the C.I. 4118 Atlas was in the C.I. collection grown by R. G. Shands at Madison in 1942. The reactions of these varieties to 3 stripe cultures are shown in table 1. Cultures C-1 and 45-2 have been used for several years in varietal trials, and are of Wisconsin origin.

The Atlas from the 3 sources reacted similarly, although the one from Wisconsin was slightly more susceptible. Atlas was resistant to C-1 and 45-2, but susceptible to GHA2. Oderbrucker was susceptible to C-1 and 45-2, but highly resistant to GHA2. Colsess was susceptible to all 3 cul-

¹ Shands, H. L., and D. C. Arny. Stripe reaction of spring barley varieties. Phytopath. 34: 572-585. 1944.

² Suneson, C. A., and Sylvia C. Santoni. Barley varieties resistant to stripe. (Note.) Jour. Amer. Soc. Agron. 35: 736-737. 1943.

tures. These results show that stripe culture GHA2 is pathogenically distinct from cultures C-1 and 45-2.—D. C. Arny, Departments of Agronomy and Plant Pathology, University of Wisconsin, Madison, Wisconsin.

Nematode Infection of Croft Easter Lilies.—A preliminary survey of greenhouses in the State of Washington during April, 1944, showed a rather general infection of Croft Easter lilies by the bud and leaf nematode, Aphelenchoides olesistus (Ritzema Bos) Steiner and Buhrer. Field examinations of lily plantings during the summer of 1944 also showed the infection to be rather general throughout areas producing this crop.

Infected plants (greenhouse forced and field grown) have strongly undercurled leaves which become bronzy and later turn brown and die. This condition had been previously observed by McWhorter¹ and named "dieback." Experience to date indicates that this symptom can be used in the field for identifying infected plants.

Microscopical examination of 155 lily plants with dieback revealed heavy infections of this bud and leaf nematode in all bronzed leaves. No other type of foliage carried infection.

Dieback plants were dug during September, 1944, and the bulbs replanted without treatment in October on soil that had never produced lilies, but had been used for many years as a chicken run. Examination of these bulbs at regular intervals following planting shows a constant infection of Aphelenchoides olesistus in all stages of development, between the growing tip (next year's foliage and bloom) and its surrounding scales. The number of nematodes per bulb has varied from 3 to 900.

These observations confirm the fact that dieback of the Croft Easter lily in Washington is caused by an infection of the bud and leaf nematode, Aphelenchoides olesistus, and that this infection is carried in the propagation stock. Experiments on phases of life history and possible control of this nematode are in progress.—Wilbur D. Courtney, U. S. Dept. Agr., Division of Nematology, Western Wash. Experiment Station, Puyallup, Washington.

Inoculations of the Evergreen Species of Prunus (Laurocerasus) with Tranzschelia pruni-spinosae.—The literature does not list any evergreen species of Prunus as hosts of the rust fungus, Tranzschelia pruni-spinosae (Pers.) Diet. Of the dozen or more evergreen species those available for inoculation were: Prunus caroliniana Ait., P. ilicifolia Walp., P. ilicifolia var. integrifolia Sudw. (P. lyoni Sorey), P. Laurocerasus Linn., P. lusitanica Linn., and P. schipkaensis Spaeth.

Prunus caroliniana and P. ilicifolia were infected by artificial inoculation with urediospores of Tranzschelia pruni-spinosae discolor Dunegan from peach and P. pumila. No evidence of infection developed on the other species.

¹ McWhorter, Frank P., S. L. Emsweller, and Philip Brierley. Suggestions for growing Easter lily bulbs in the Pacific Northwest. Ore. State Exp. Sta. Cir. 339. 1944.

The methods used were as follows: The foliage of the plant to be inoculated was sprinkled with water, and the inoculum on the rust-infected leaves was placed in contact with the healthy leaves. The inoculated plants or plant parts were enclosed in a moist chamber or in a cellophane bag. In other experiments the plant was enclosed in a cheesecloth chamber (Fig. 1,

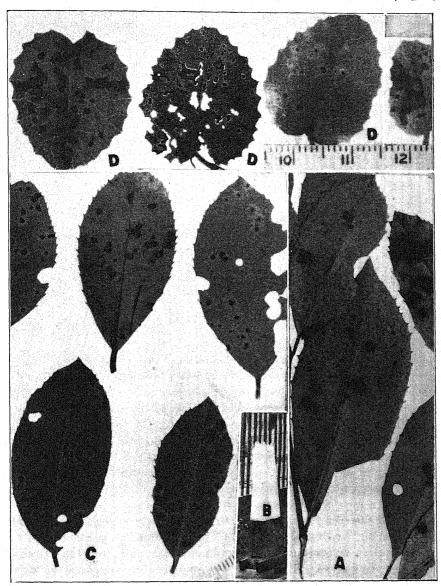


Fig. 1. Rust infection on certain evergreen species of *Prunus:* A, natural infection on *P. caroliniana;* B, a cheesecloth chamber used in the inoculation experiments; C, results of artificial inoculations on *P. caroliniana* (note millimeter scale near bottom). The infected areas had fallen out or were about to fall out. D, results of artificial inoculations on *P. ilicifolia.*

B) which was kept moist by a cheesecloth wick from an overhead vessel of water. In some other experiments the leaves on a detached twig were inoculated, and the stem was inserted in moist sand in a moist chamber. Satisfactory infection was secured by all methods.

Definite circular spots, 1–5 mm. in diameter (Fig. 1, C), developed on the leaves of *Prunus caroliniana* in about 3 weeks. The center of the spot was dark brown, surrounded by a greenish yellow zone. The tissues collapsed and a few urediospores of the fungus developed on these spots. The collapsed areas soon broke away from the healthy tissue, leaving a shot hole or, often,

a ragged appearance to the leaf. The spots frequently coalesced.

Infection on Prunus ilicifolia and P. ilicifolia var. integrifolia (Fig. 1, D) was similar to that on P. caroliniana. There was at first a yellowing of the infection points which gradually darkened and became brownish spots 2-3 mm. in diameter. The brownish areas did not enlarge but became surrounded by a yellowish-green zone. There was a tendency for the infected tissue (the spots) to break away from the healthy tissue, but the shot holes were not so frequent as in P. caroliniana. Fruiting of the fungus was sparse but some typical urediospores formed on some of the spots. Infection usually occurred on the younger leaves.

It seems apparent, in both species, that the urediospores of the rust fungus must come from some more susceptible species of *Prunus* and that

the fungus probably can not perpetuate itself on these hosts.

Smith and Cochran, in their description of rust on the species of *Prunus* native to California, listed *Prunus ilicifolia* as not attacked by the rust fungus, and natural infection has yet to be observed.—CLAYTON O. SMITH, University of California Citrus Experiment Station, Riverside, California.

Polyporus Versicolor on Asiatic Chestnut.—Asiatic chestnut trees for ornamental and orchard purposes are now listed for sale by a few seed houses and nurseries. They may become of considerable economic importance in the near future. Thus the susceptibility of these trees to pathogenic fungifound in America is a matter of general interest to plant pathologists.

Some Asiatic chestnut trees have been growing since 1931 at the Syracuse Experiment Station of The New York State College of Forestry, Syracuse, New York, in an area where native chestnut once grew. While making routine observations of the trees in the autumn of 1944, sporophores of *Polyporus versicolor* Fries were discovered on one small tree. The sporophores had emerged from live bark covering a somewhat enlarged localized area on each of two branches (Fig. 1). Rabbits had gnawed the base of the tree several years previous and these wounds may have been the source of entry for the fungus. The typical spongy white rot associated with this fungus was present through the heartwood of the trunk and branches.

Polyporus versicolor, commonly associated with the decay of sapwood, is known to cause decay of the heartwood in living catalpa, especially pollarded Smith, Clayton O., and L. C. Cochran. Rust on the California native prune. Phy-

topath. 29: 645-646. 1939.

catalpa commonly used for ornamental purposes. The fungus has also been reported to cause heartrot of black cherry, London plane, apple, pear, and plum. Destruction of the heartwood weakens the affected trees and makes them especially susceptible to wind, snow, and ice damage. Consequently it is important for those who care for orchard and ornamental trees to be

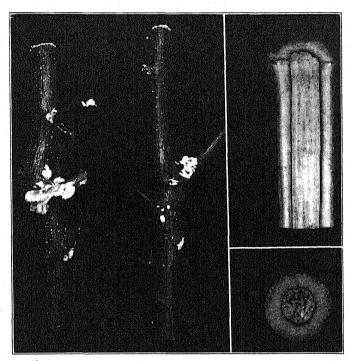
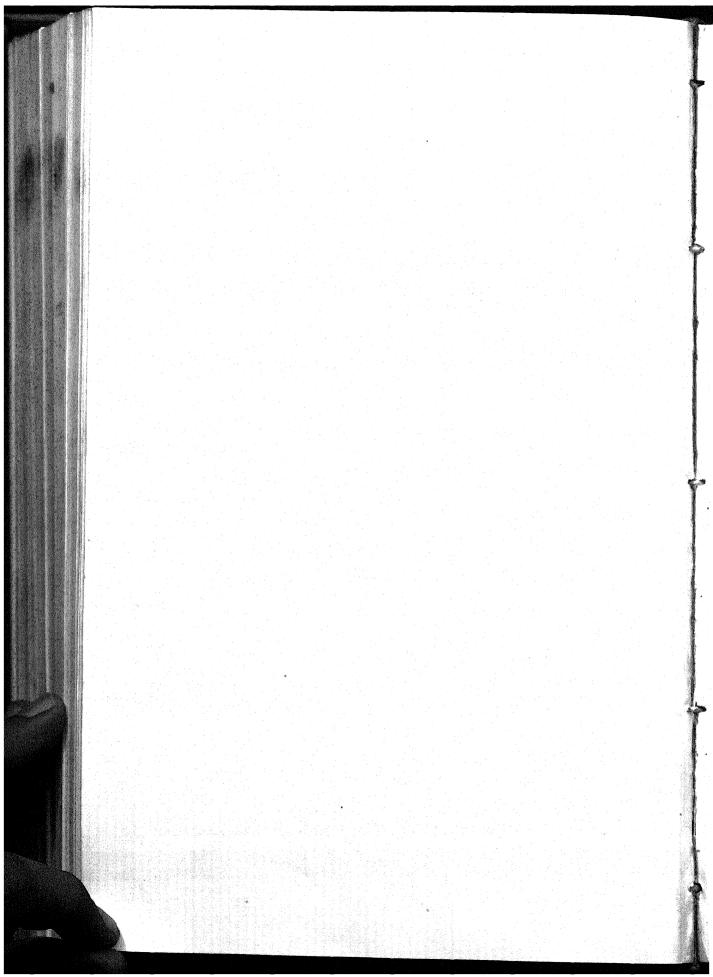


Fig 1. Polyporus versicolor causing decay of living Asiatic chestnut.

familiar with the fungi that cause heartrot, in order that proper bracing and cabling can be done before breakage occurs in the infected trees. Thus the association of *Polyporus versicolor* with heartrot of Asiatic chestnut is of interest to tree experts as well as plant pathologists.—RAY R. HIRT and J. L. LOWE. The New York State College of Forestry, Syracuse, N. Y.



ARTHUR HENRY REGINALD BULLER 1874-1944

W. F. HANNA, C. W. LOWE, AND E. C. STAKMAN

Professor Arthur Henry Reginald Buller was born in Birmingham, England, August 19, 1874, and died in Winnipeg, Manitoba, July 3, 1944, of a brain tumor. During a long and uninterrupted career as an investigator and teacher his contribution to science, particularly in his chosen field of mycology, was prodigious. And yet, despite his extraordinary ability and industry, he left much unfinished work, a possibility about which he had often expressed concern.

After obtaining the B.Sc. degree from the University of London in 1896, Buller was awarded an 1851 Exhibition Scholarship which enabled him to study under Pfeffer in Leipzig, where he was granted the Ph.D. degree in 1899. He also studied with Hartig in Munich and later spent some time at the Marine Biological Station at Naples. Buller often paid tribute to the stimulating influence of both Pfeffer and Hartig. He reminisced especially about his field trips with Hartig and cherished recollections of his close association with this pioneer in the field of forest pathology.

From 1901 to 1904 Buller was lecturer in botany at the University of Birmingham and in the latter year went to the University of Manitoba at Winnipeg as professor of Botany, where he remained until his voluntary retirement as professor emeritus in 1936. When asked how he happened to go to Winnipeg, Buller said that he had seen in some literature about Canada a description of "Winnipeg, The Mushroom City" and had immediately realized that it would suit his tastes and interests so nicely that he decided to cast his lot with the thriving young metropolis of the Canadian prairies. And there he taught his students and immersed himself in his researches on fungi.

Although he crossed the Atlantic 65 times, in order to spend his summers in England, Buller formed a strong attachment for Canada and worked hard for the development of his University and the Dominion Laboratory of Plant Pathology, established originally in 1923 as the Dominion Rust Research Laboratory. The summer of 1939 found Buller in England, but early in September of that year he came to New York to attend the meetings of the International Microbiological Congress. At the close of the Congress he decided, because of the difficulties of ocean travel, to return to Winnipeg where he remained until his death except for occasional absences for lectures and special courses at various American Universities. Had it not been for the war, Buller probably would have taken up permanent residence in England and there continued with his writing and research.

As a teacher Buller was a perfectionist. He prepared lectures and laboratory materials with meticulous care and attempted to make every lecture a finished performance. Likewise, he considered it an obligation to



ARTHUR HENRY REGINALD BULLER 1874–1944

make each laboratory period a really valuable experience for the students. He was as exacting of himself as of his students. Slovenliness of any kind, mental or physical, was abhorrent to him. Orderliness and preciseness were among his cardinal requirements. To Buller the lecture room and laboratory were sacred to science, and he tolerated no intellectual inattention or personal misbehavior. Many students have testified to the disciplinary values of his courses. And yet Buller himself was so interesting, so genuinely enthusiastic in his teaching, and had to so high a degree the faculty of making botany interesting and significant that serious students evaluated his teaching not only as an intellectual discipline but also as a stimulus to the expansion of mental horizons. This is the consensus of a random sample of former undergraduate students after a lapse of time had given them perspective on educational values.

Buller's attitude towards post-graduate study and research was profoundly affected by the environment in which he moved during the early years of his scientific career. As a young science student he became absorbed in Darwin's great work on the Origin of Species and was deeply influenced by Huxley's brilliant advocacy of the theory of evolution and his defense of the scientific method. In this stimulating environment science was revealed to Buller as something more than a métier; it was a never-ending search for truth, a thrilling adventure of mind and spirit in new fields of knowledge, with fresh vistas ever stretching beyond the horizon; it was for him a way of life. At the time Buller went to Germany to study with Pfeffer and Hartig, the wholesale application of science to industry and agriculture, necessitating as it has done the training of great numbers of specialized scientific workers, had scarcely begun. The number of graduate courses available to research students was strictly limited, and the relationship between professor and student was much more intimate than is possible in the scientific laboratory of today. The high degree of specialization which characterizes present-day scientific study was unknown in the late nineties and indeed, in this regard, it may be recalled that Buller's early scientific training and interests covered the fields of zoology and geology, as well as that of botany. With such a background, then, it was but natural that Buller viewed with a mixture of regret and understanding the growing tendency towards specialization in the various fields of applied science. In later life when his work was widely known and his position in the scientific world was firmly established, he might have built up a large graduate school, but he preferred instead to give his attention to a relatively small number of students whose work he could closely supervise, while at the same time continuing with his own writing and research.

Buller's relationship with his graduate students was always warm and intimate. He was a friend and counselor as well as a stimulating teacher. His enthusiasm was contagious, his energy untiring, his way of life both unconventional and unpredictable. A confirmed bachelor, he was in every sense of the word "unattached." If some interesting experiment was to be

attended to or if a fruitful new idea came to his mind he might appear in the laboratory at five or six o'clock in the morning and work furiously throughout the day or even until after midnight, stopping only for a lunch of tea and biscuits at his desk and perhaps forgetting entirely about dinner. But when the task was accomplished, Buller had the happy faculty of being able to relax completely and he might then indulge in his favorite recreations—a game of billiards, a cigar, a turn at the piano, a half hour with Shake-speare, Keats or Shelley, a good novel, an occasional movie, or a stroll among the trees and flowers. While always tolerant of others he perhaps never fully understood the predicament of more orthodox individuals whose way of life seemed to conform to a fixed pattern and whose comings and goings were regulated by habit rather than by the task at hand.

Buller was essentially a student of Nature and of living things. He was in no sense a systematist, although his knowledge of plant and animal species was remarkably complete. His chief interest lay in elucidating the living processes of organisms, their methods of reproduction and dissemination, and their relationships to environment. To him laboratory studies were merely a means to this end and he never failed to relate his laboratory findings to his observations in the field. Not a few of his most important laboratory discoveries were suggested by field observations. Trained in a period when botanists relied mainly on the microscope, the pencil, and the free-hand section, he regarded with some suspicion the conclusions which were occasionally drawn from the study of fixed and stained microtome sections. He was adept at cutting free-hand sections of living material and from them he made numerous and accurate sketches later to be converted into the beautiful line drawings with which his Researches and scientific papers are so copiously illustrated. He worked rapidly, his powers of observation were amazing, and he never lost sight of the possible significance of the most trivial observation.

Although endowed with a keen imagination, the imagination of an artist, Buller never allowed his imaginative faculties to dominate his reason. His fund of ideas was seemingly inexhaustible and his students would sometimes almost despair when, in the course of a brief conversation, he might suggest a multitude of interesting experiments which nevertheless would require months to carry out. He speculated freely, but he was always eager to submit his hypotheses to the acid test of experimentation.

In the course of his scientific career Buller developed a clear, easy, and forceful style of writing. He was ever willing to assist his students in the preparation and revision of their papers and, while avoiding undue interference with the individual's methods of expression, he nevertheless insisted upon the observance of certain fundamental rules. Accuracy and clarity were for him the prime requisites of good scientific writing. He made a practice of reading his own manuscripts aloud to his students and invited their criticism and comment. To the novice who sought in vain a precise word or a nicely turned phrase he gave the encouraging assurance that he

himself in his student days had experienced no less a difficulty. And yet to all who knew him it was evident that the ease with which he expressed his thoughts in both verse and prose could not have been acquired by practice alone, but stemmed from a fine natural feeling for the choice and meaning of words.

Perhaps the most vivid impressions which graduate students retain of Buller are his enthusiastic devotion to science, his love of Nature, his kindliness, and his generosity to those who needed a helping hand. Above all he was an English gentleman in the finest sense of the word. Although he came to have a deep attachment for Canada, where he spent nearly forty years of his life, he always remained at heart an Englishman and the last years of his life were saddened by the destruction wrought by war in his native land which ever was for him:

This precious stone set in a silver sea— This blessed plot, this earth, this realm, this England.

Research was a passion with Buller: Few men could have had for it such single and deep devotion. He worked to the limit of his strength and sometimes beyond. Buller reduced the mechanics of living to its simplest terms in order that he might be completely free to devote himself to his science, and his attainments are evidence that he must have worked with virtually maximum efficiency most of the time. He often said that he had a poor memory; but his rich store of information belied his assertion. Native curiosity, acuteness of observation, and everlasting persistence led him to discover many obscure and apparently insignificant facts. But nothing in Nature was insignificant to Buller. Through his powers of synthesis he wove many of these apparently insignificant facts into highly significant principles. He was sometimes twitted with being a teleologist; if his viewpoint was teleological, it certainly paid dividends, both in his researches and in his ability to dramatize the life of the fungi. He was, of course, not a teleologist in the invidious connotation of the word: rather he looked always for relationships between phenomena and for the utility of structures and processes. He reasoned that there must be underlying causes for the phenomena which he observed and he was not satisfied until he found them.

Researches on Fungi, six volumes filled with a multitude of interesting and important facts and ideas about fungus behavior, presented in an easy, lucid style, constitute a monument to Buller's genius. He made fascinating stories out of spore production, spore liberation, and spore dissemination. He studied sex, so-called social organization, hyphal fusions, protoplasmic streaming, and nuclear associations. His pen pictures were extraordinary in a double sense; he was an artist, verbally and graphically. But Researches on Fungi are not only interesting; for they contain much that is valuable in a practical way. His studies on spore formation, liberation, and dispersal pointed the way, even though indirectly, to a better comprehension of some of the factors affecting the development of plant-disease epidemics. And yet, his books are a treasure of information quite apart

from any values other than those that satisfy an inquiring mind or cause it to wonder at the marvels of structure and function of some of the most fascinatingly mysterious of all living organisms. For Buller himself often marvelled at the wonderfully nice adaptations and adjustments that enable mushrooms to crowd prodigious numbers of spores on their hymenial surface and then ensure their liberation. Buller was a scientist; therefore he studied as closely as possible the mechanisms involved. But he was a philosopher too; and he wondered and speculated about the nature of the genes that directed the building of the structures and the functioning of the mechanisms. And this intense desire not only to know what happened but also why it happened impelled him to delve more deeply than most into the intimate life of the fungi. With infinite pains and infinite patience he accumulated a vast store of knowledge about things that had been taken for granted. For Buller took nothing for granted.

Professor Buller was in great demand as a lecturer: he gave many individual lectures and held a number of special lectureships in the United States. He was Norman Wait Harris Foundation lecturer at Northwestern University in 1927, summer lecturer at Louisiana University in 1941, Hitchcock professor at California in 1942, and in the same year Schiff Foundation lecturer at Cornell.

Scientific affiliations included membership in the American Phytopathological Society, Botanical Society of America (president in 1928), British Association, British Mycological Society (president in 1913), Canadian Phytopathological Society (president in 1920), and in the Society for Psychical Research. He was associate member of the Société Royale de Botanique de Belgique, corresponding member of Société Botanique Neerlandaise, honorary member of the Indian Botanical Society, and Fellow of the Royal Society of Canada (president in 1928). In 1929 he was elected Fellow of the Royal Society of London.

The honorary degree of Doctor of Laws was conferred upon him by the University of Manitoba in 1924, by the University of Saskatchewan in 1928, and by the University of Calcutta in 1938. Pennsylvania University granted him the degree of Doctor of Science in 1933. He was recipient of the Flavelle medal from the Royal Society of Canada in 1929, the medal of the Natural History Society of Manitoba in 1936, and the Royal Medal of the Royal Society in 1937.

Buller's interests were many and varied. He loved to stroll in the woods and fields, sometimes to make professional observations, but quite as often to commune peacefully with Nature. He was fond of birds and noted their habits with keen interest. His days were usually spent in teaching, writing, and research, but in his leisure moments he was a discriminating reader of good prose and verse. Endowed with the gift of expressing his thoughts in verse as well as in prose, he left behind a sizeable collection of poems, rhymes, and limericks, many of which have real merit. Always a lover of books, he gathered together a fine selection of botanical works, including

many rare and interesting volumes on the fungi. His recreations included billiards, which he played with skill and enthusiasm, playing the piano for his own amusement or to entertain his friends, and occasional tricks of legerdemain. Withal, he had a good sense of humor and a kindly nature which, with his little quirks of character, made him always an interesting and entertaining companion.

In his chosen field of science, Buller attained preeminence. The last of his vivid lectures has been given, but his published works remain as a remarkable record of achievement of an extraordinary scientist and as an inspiration for present and future students of the fungi.

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THE EFFECT OF RUGOSE MOSAIC ON THE YIELD OF POTATOES

J. G. BALD (Accepted for publication December 27, 1944)

DESCRIPTION OF THE EXPERIMENTS

In two field experiments at Canberra some potato plants of the variety known in Australia as Early Carman (probably Green Mountain), grown from tubers thought to be healthy, were found to be infected with rugose mosaic. For the original purpose of these experiments, periodical measurements of leaf area were taken on each plant (1, 3), and also a record of the final yield. Data of this sort provided information about the growth of the foliage, and about the relation between leaf area and yield of diseased and normal plants.

The plants were grown in the same poor sandy loam in different seasons, one lot during the early portion of the season 1941–42, and the other in the later portion of 1942–43. The original experiments have been described elsewhere (1, 3, 5).

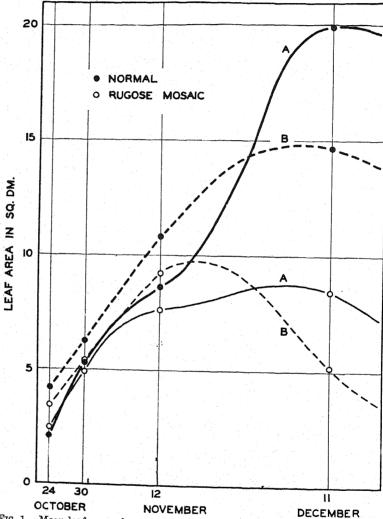
In both instances the conditions of growth were far from optimal. The weather was hot and dry, and the light intensity high. The plants had to be watered to maintain growth. In the first experiment, during the later stages of development the plants received barely enough water to allow them to finish their growth. In the second experiment, from the time of flowering onwards, the foliage of the plants was damaged by larvae of the potato moth. However, there is no reason to suspect that these conditions seriously affected the comparison between the diseased and normal plants. The environment in which the plants grew may be summarized as one in which the efficiency of photosynthesis was likely to be high and the absorption of water and nutrients from the soil was likely to be the limiting factor for growth, even of diseased plants.

In the 1941-42 experiment there were 10 plants infected with rugose mosaic, and 20 plants, in the same small plots, that were normal throughout the season. These fell, according to their position in the experimental area, into equal groups of 5 diseased plants and 10 normal. The "normal" plants contained virus X, and the diseased plants a mixture of viruses X and Y. The comparison was thus not between diseased and completely healthy material, but between plants carrying latent and obvious infection.

In the experiment of 1942–43, six normal Early Carman plants and two infected with rugose mosaic were studied in detail. The development of the individual leaves on the main and axillary shoots was followed until the maximum leaf areas were attained, and estimates of the total leaf area were obtained throughout the whole period of growth from emergence to senescence (3, 5). Owing to the conditions of temperature and daylength late in the season when the plants were grown, the total period of development was only about two-thirds as long as in 1941–42.

RESULTS

In figure 1 is shown for the 1941–42 experiment the leaf area of the two groups of diseased and healthy plants from October 24 to December 20. They reflect differences in soil moisture in the portions of the experimental area where they grew. The growth of group A was checked soon after the



Fro. 1. Mean leaf area of potato plants infected with rugose mosaic (viruses X and Y) and "normal" plants (containing virus X). There were two groups of plants, A and B, from different parts of the experimental field. In each group were five diseased plants and ten healthy. From 1941-42 experiment.

inception of flowering, but later, after being irrigated, the normal plants increased rapidly in size, and reached a greater maximum leaf area than the normal plants in group B. The diseased plants of group A also made fresh growth, but at the same time they lost some of their lower leaves. The leaves

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shrivelled in the manner usual, during the later stages of development, with mosaic-infected plants of this variety. The effect of the loss of leaves is clearer in the growth curve for diseased plants in group B, the leaf area of which reached a clear maximum and then declined.

During the earliest stages of growth, these diseased plants revealed little evidence of infection and increased in size at almost the same rate as the normal plants. Later, as symptoms became evident, the growth rates declined in comparison with those of the normal plants. By November 12, the mean leaf area of the diseased plants was 14 per cent below that of the normal plants, and the difference was significant. In the combined groups, A and B, the mean of the maximum leaf areas reached by the diseased plants was little more than half the maximum for the normal plants.

The total yields in table 1 show differences between diseased and normal plants of the same order as for the mean maximum leaf area. The ratio

TABLE 1.—Yield of Early Carman plants infected with rugose mosaic (viruses X and Y) and normal plants (infected with virus X); 1941-42 experiment

		Yield in					
Groups and state No. of plants plants	Tubers below 2 oz.		Tubers above 2 oz.		Total		gm. per sq. dm. of leaf
	No.	Wt.	No.	Wt.	No.	Wt.	area
A. Diseased 5 Normal 10	2.2 1.9	64 72	1.6 3.1	115 265	3.8 5.0	179 337	8.8 8.7
B. Diseased 5 Normal 10	$\frac{3.0}{2.7}$	104 96	$\begin{array}{c} 1.4 \\ 3.0 \end{array}$	97 299	$\frac{4.4}{5.7}$	201 395	$10.7 \\ 11.2$

between maximum leaf areas and yield in the two blocks varied considerably, but the variation arose mainly from the different forms of the foliar growth curves. A better estimate than the maximum leaf area of the efficiency of the leaves in the formation of tubers is given by the integration of the values for leaf area over the period from the inception of flowering (which may also be taken as the inception of tuberation) until the last estimate of leaf area, i.e., for approximately six weeks from November 10. The simplest method of integration is to find the mean leaf area over this period. Values for the yield per sq. decimeter of mean leaf area are in table 1. Equal areas of leaf on diseased and normal plants produced very nearly the same weight of tubers.

Infection with rugose mosaic caused a greater fall in the weight of total yield than in the number of tubers. The yield of tubers below marketable size was about equal for diseased and normal plants, and therefore the loss in total yield due to virus Y was less than the loss in marketable yield. The loss in marketable yield was about 63 per cent.

Diseased plants in the second (1942-43) experiment developed symptoms immediately on emergence, and were severely dwarfed. This is characteristic of plants from infected tubers that have been sprouted, greened, and

held for a considerable time at fairly warm temperatures. In the 1942–43 experiment, the mean leaf area of the two infected plants was little more than one-tenth the mean leaf area of the six normal plants.

As the growing period in this experiment was only about two-thirds that of the plants in the earlier experiment, the leaf area during a period of 30 days instead of six weeks after the beginning of flowering was integrated by taking the mean. The yields were weighed to the nearest five grams. The results for the individual plants, six normal and two diseased, are in table 2.

As in the previous experiment, there was no demonstrable difference between diseased and healthy plants in the yield per sq. decimeter of leaf area. Amongst the plants from numbers 7 to 14, which included the two diseased plants, the variation in the values was negligible. The healthy

TABLE 2.—Mean leaf area over a period of 30 days from the initiation of flowering, and total yield of six normal Early Carman plants, and two infected with rugose mosaic; 1942-43 experiment

	Position in Mean leaf -					Yield					
State of plants		ition row	in	ε	Mean leaf irea (sq. d		Phones and the	(gm.)			(gm./sq. dm.)
Normal		3			10.6			115			10.9
		6			28.8			330			11.1
		8			17.9			290			16.2
		13			18.6			205			16.4
		14			29.0			470			16.2
		15			19.2			270			14.1
Diseased		7			1.76			30			17.0
		10			4.65			70			15.1

plants 3 and 6, and, to a lesser extent, 15 gave lower values, probably because of a deficiency of moisture in the soil where they grew.

The results of the leaf area measurements agree with those of Stone (7) made on similar diseased material, and need not be given in detail. As the leaf area measurements were carried beyond the stage of development studied by Stone, several points may be added to her description of the foliar growth. Even on the diseased plants of the 1941–42 experiment, on which symptoms were slow to appear, there was not the usual number of axillary shoots. Those that did form were small. The reduced axillary growth and the premature shrivelling of the lower leaves were the main causes of the reduced leaf area of diseased plants. In the 1942–43 experiment, the diseased plants produced practically no axillary shoots, and the leaves on the main axes were very small. The loss of the basal leaves was hastened by the attack of potato moth larvae. However, the normal plants were attacked in a similar manner (5).

DISCUSSION

Among the potato plants diseased with rugose mosaic there was a number of indications of restriction in the plants' supplies of available protein.

Firstly, there was the reduction in leaf area associated with infection. In healthy plants of related genera, leaf area is a fair measure of protein content in the pre-senescent stages of growth (6). Presumably the manufacture of virus protein replaced not only the manufacture of reserve protein (9), but also the manufacture of some of the cytoplasmic protein necessary for the formation of new centers of metabolism in the leaf tissues. The reduction of axillary growth on diseased plants in the first experiment and its almost complete absence in the second suggest that the available protein in diseased plants was insufficient to support, in addition to tuber formation, the development of tissues from secondary growing points. The premature shrivelling of the lower leaves suggests that even the cytoplasmic protein they contained was hydrolyzed to maintain growth elsewhere, or for the manufacture of additional virus protein (8).

On the other hand, the functional leaf area measured during the presenescent tuber-forming stage of development appeared as efficient in the formation of tubers on plants diseased with rugose mosaic as on "normal" plants containing only virus X. Apparently there was no inhibition of translocation in the diseased plants, and the efficiency of the centers of metabolism actually formed in the leaves of diseased plants, in so far as it was reflected in the formation of tubers, was not further reduced by the imposition of virus Y on the original infection with virus X.

These results, on the surface, contradict those obtained by Stone (7), who found that mosaic-infected plants produced a smaller weight of tubers per unit of leaf area than the normal control; but there are points of difference between the American and Australian experiments that make a direct comparison of results difficult. The yields obtained by Stone were not mature yields, and it is probable that the course of tuber development is different in normal and mosaic plants. Also, leaf areas were calculated only for the earlier phases of development, and the means were partly derived from daily values obtained before the stage at which tuberation begins. If, in the experiments described here, the final yield is divided by the mean leaf area over a period equivalent to that used in Stone's calculations, the yield per sq. decimeter of leaf area is considerably greater for normal than for diseased plants. For example, in the 1941–42 experiment, the mean figures are 43.1 and 26.8 gm. per sq. decimeter.

There thus appear to be two levels in the reduction of yield due to the mosaic diseases of the potato. At the first level, represented by infection with average strain mixtures of virus X, the virus appears to multiply in the host at the expense of reserve proteins only, as the foliar portions of the plant grow to the normal size (2, 4). Loss of yield is presumably due to the inability of the plant to hydrolyze the virus protein for translocation to the tubers. At the second level, represented by plants infected with rugose mosaic, the competition exerted by the metabolism of virus protein against the normal protein metabolism is sufficient not only to replace reserve protein by virus protein, but also to replace protein needed for the establish-

ment of new centers of metabolism. This results in a smaller leaf area on diseased plants, and an enhanced reduction of yield.

SUMMARY

In two experiments, leaf areas were measured on potato plants infected with rugose mosaic, due to viruses X and Y, and on "normal" plants containing virus X. Final yields were also measured. The reduction in yield due to virus Y was proportional to the reduction in leaf area measured during a period after the initiation of tuber formation. The yield of tubers over 2 oz. in weight was reduced by infection with virus Y more than the total yield, because the reduction in numbers of tubers was proportionately less than the reduction in leaf area.

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH, DIVISION OF PLANT INDUSTRY, CANBERRA, A. C. T., AUSTRALIA.

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VIRUS C FROM AN OLD AUSTRALIAN VARIETY OF POTATO

J. G. BALD AND D. O. NORRIS (Accepted for publication December 27, 1944)

INTRODUCTION

In November, 1938, studies were begun on a virus that had been isolated several times from an old Australian potato variety, Brown's River (1). Its properties and reactions corresponded with those given in the meager descriptions of virus C then available (2, 6). As the Brown's River virus was studied in detail, it was found also to be closely similar to virus Y. Apart from its reactions on the standard varieties of potato, the only property found to differentiate it from virus Y was the inability of the aphid, Myzus persicae Sulz., to transmit it to healthy plants of susceptible varieties, under conditions in which virus Y was readily transmissible.

Even this distinction was not complete, as transmission of the Brown's River virus by Myzus persicae was recorded in two instances. On the other hand, although previous infection with virus Y usually prevented potato plants from reacting to the Brown's River virus with characteristic symptoms, there were instances in which we could not be sure that infection with virus Y had given complete protection. The evidence on the identity of the Brown's River virus was thus somewhat confused. Further experiments on its transmissibility and its relationship to virus Y were planned, but could not be made at the time. Since then, the published results of other workers (3, 5) and some more recent work at Canberra have removed doubts that the Brown's River virus is virus C, and that virus C must be considered a strain of virus Y.

SOURCE OF THE VIRUS

The Brown's River potato¹ is a late variety with reddish purple tubers and a vigorous habit of growth. The two commercial stocks examined were almost entirely infected with virus C or virus Y. Infection of Brown's River with virus C is often not detectable in the field, but in the greenhouse it causes a slight veinal mottle and rugosity of the leaves. Sometimes in the greenhouse the mottle is masked, and the only symptom is a slight rugosity, that can be distinguished by comparison with healthy plants. This comparison has been made possible by the isolation of virus-free stocks of Brown's River from single plant selections made by Dr. R. A. Scott of the Tasmanian Department of Agriculture.

SYMPTOMS ON POTATO

Brown's River has field resistance or immunity from virus X, and uncontaminated samples of virus C have readily been obtained from it by

1 Other names applied to this variety were Brown's River Black, Circular Head, and Redskin. It was the principal maincrop variety in Australia during the nineteenth century.

inoculation to tobacco and other susceptible hosts. Although Bawden (2) originally stated that "virus C has not been transmitted to any plants by needle inoculation," Dykstra (7) found mechanical transmission to tobacco very easy by the methods he used, and Cockerham (4) states that mechanical

TABLE 1.—Summary of the symptoms on 11 varieties of potato inoculated or grafted with material containing Brown's River virus

Variety and means of trans- mission	Symptoms
President (graft)	Top necrosis.
(inoculation)	Necrotic local lesions.
Up-to-Date (graft)	Top necrosis.
(inoculation)	Necrotic local lesions (Fig. 1).
Arran Victory (graft and in- oculation)	Very mild mosaic.
Epicure (graft)	Top necrosis, not always severe enough to kill the plant rapidly. From the tubers small plants were pro- duced which became necrotic and died when they were a few inches high, or developed a dwarfing mosaic disease.
(inoculation)	Necrotic local lesions, and occasionally systemic symptoms as above.
Katahdin (graft and inoculation)	A rather indefinite mottle in the current season, some- times a certain amount of necrosis and shrivelling of the lower leaves; in the following season definite and characteristic crinkling and veinal mottle.
Seedling 41956 (graft)	Similar to Katahdin but more severe; no current season necrosis was observed.
Carman (graft)	Top necrosis.
" (inoculation)	Local necrosis, necrotic streaking of petioles and stem, top necrosis.
Brownell (graft)	Only one plant was grafted. No current season symptoms were observed, but in the following season a veinal mottle and rugosity developed, necrotic streaking on the undersides of the veins and shrivelling of the lower leaves.
Snowflake (graft)	Top necrosis; in the second season dwarfing, severe crinkle, necrosis and early death.
" (inoculation)	Local necrosis and retention patterns, caused by the loss of chlorophyll except in restricted areas of the leaf. Systemic symptoms were semi-necrotic, spreading slowly to the growing tip.
Bismarck (graft)	Severe mottle, rugosity, thin necrotic streaks on the
	undersides of the veins, shedding of the lower leaves until only a terminal tuft remained. In the second season, severe mottle, rugosity and some necrosis.
Delaware (Earliest of All) (graft)	Top necrosis beginning as necrotic spots or a necrotic network on the veins of the youngest leaves, streak- ing of the stems and collapse.

transmission to potatoes reacting without necrosis is obtainable. At Canberra, the Brown's River virus was transmitted both to certain varieties of potato and to other hosts by mechanical inoculation with a glass spatula and carborundum powder. For varieties of potato that did not react by necrosis, this method appeared reasonably effective, but not always so effective as similar inoculations with virus Y.

The varieties inoculated or grafted from the original Brown's River material were the four English test varieties, President, Up-to-Date, Arran Victory, and Epicure; the American varieties, Katahdin and U. S. seedling 41956; and the five varieties, which, apart from Up-to-Date, are most widely grown in Australia, Carman (possibly Green Mountain), Brownell (Adirondack), Snowflake, Bismarck, and Western Australian Delaware (probably Earliest of All). Their reactions are in table 1.

OTHER SOLANACEOUS HOSTS

Four common solanaceous hosts have been tested for their reaction to the Brown's River virus; tobacco, variety Hickory Pryor, Nicotiana glutinosa, Datura stramonium, and pepper. Of these, N. glutinosa gives the most

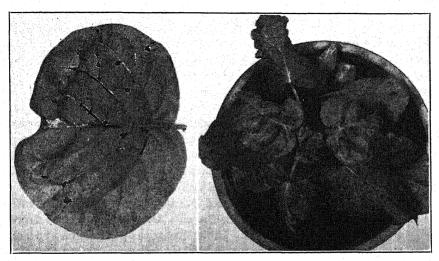


Fig. 1. Left. Necrotic lesions produced on inoculated leaves of Up-to-Date potato by the Brown's River virus. Right. Fully developed symptoms of the Brown's River virus on Nicotiana glutinosa.

characteristic reaction. Occasionally, 5 or 6 days after inoculation, indefinite yellow local lesions may be produced. Under normal conditions, after an incubation period of between 7 and 12 days, vein-clearing occurs, and within another week the areas between the veins balloon upwards, and a mottle develops consisting of vein-banding and a loss of color in the interveinal areas (Fig. 1). The plant grows on, but is stunted, and distortion of the younger leaves may be severe. Samples of virus Y inoculated in parallel series as a rule gave similar but less severe symptoms. The symptoms on tobacco are almost indistinguishable from those of virus Y. On Datura no infection has been produced by inoculation or grafting. On pepper infection occurs, but symptoms do not appear until about five weeks after inoculation. They consist of a mild but characteristic mottle; yellow-green areas extend from the main veins, vein-banding or green islands develop sparsely along the veins, and the mottle may be outlined by light bronze lines. The leaves may roll upwards, and become yellowish green.

The addition of virus X to the Brown's River virus in tobacco or *Nicotiana glutinosa* generally produces double symptoms like those characteristic of the virus X-virus Y mixture, *i.e.*, yellow vein-clearing, and more or less necrosis according to the strain of X used. In at least one instance, however, when the Brown's River virus was present in tobacco and virus X was added, the resultant symptoms were mild. In other instances, in which a mixture of the two viruses was inoculated to tobacco, the usual double-virus symptoms did not develop very clearly, and those due to virus X were predominant.

Six hosts outside the *Solanaceae* were inoculated with the Brown's River virus without producing any infection. These were cucumber, zinnia, and cowpea (which react to cucumber mosaic), pea, French bean, and broad bean.

INSECT TRANSMISSION

The only insect tested as a vector of the Brown's River virus has been the aphid, Myzus persicae, the most efficient vector of virus Y. Early experiments in which the aphids were fed on the shoots of sprouted tubers were negative, but so also were parallel tests with virus Y, although leaf roll was transmitted easily. Sprouting tubers are a poor source of virus Y, and probably of virus C. An indication that M. persicae might be a vector of the Brown's River virus was obtained in one instance when aphids feeding on an infected plant were transferred to President. On one leaf of one plant a necrotic lesion developed and spread down a main vein, eventually killing the leaf. Systemic infection did not occur.

In other experiments aphids were fed on diseased plants of potato varieties with a non-necrotic reaction to the Brown's River virus, and transferred to *Nicotiana glutinosa* and tobacco. In the first experiment of this type, one *Nicotiana glutinosa* of five inoculated became infected. Other transfers gave negative results.

In all, 16 transfers were made to varieties of potato reacting to the virus with necrosis, 27 to varieties with a non-necrotic reaction, 16 to *Nicotiana glutinosa*, 15 to tobacco, and 10 to pepper, a total of 84. About 20 aphids per plant were used for each one. Only in the two instances described did infection occur.

PHYSICAL PROPERTIES OF THE VIRUS

Dilution End-point

The results of four experiments are shown in table 2. The first two were performed at different times with extracts of recently infected plants of *Nicotiana glutinosa*, the last two with leaves from tobacco plants that had been infected for a number of weeks. The results show the usual diminution of concentration that occurs as infected plants age. The inoculum for the fourth experiment was extracted from the leaves by the chemical method Stanley used for the preliminary stages in the isolation of bushy stunt-virus (9). The reason for the failure of infection at the 1 in 5 dilution may have been the high concentration of phosphate in the inoculum.

These are much higher dilution end-points than are recorded by Kenneth Smith (8) for virus Y. Under our conditions, however, the dilution end-point of virus Y is of the same order as that of the Brown's River virus. This may partly be the result of more efficient methods of inoculation, but it is probably also a reflection of the high concentrations of virus that normally develop in host plants under the high light intensity and temperatures in the greenhouse at Canberra. The Brown's River virus also retained its infectivity in vitro at higher temperatures and for longer periods than is recorded for virus Y, but parallel experiments with virus Y under our conditions gave similar high values.

TABLE 2.—Dilution of the Brown's River virus. Number of plants, of eight inoculated, infected at the dilutions shown

77			Dilution				
Experiment —	Undil.	5	100 10	00 10,000	100,000		
1 2 3 4a	8	8 0	8 6 2	8 4 8 4 1 0	0		

 $^{^{\}rm a}$ Tobacco leaves from the same lot of plants as in Experiment 3 were frozen for five days, ground up with a small quantity of a 50 per cent solution of $\rm K_2HPO_4$ and the juice was expressed through muslin.

Heat Inactivation

Juice was obtained from infected plants of *Nicotiana glutinosa* or tobacco, diluted 1 in 5, and divided into 10-cc. lots, which were held for 10 min. at different temperatures. Tests were made both on untreated juice and on juice that had been clarified by spinning in a centrifuge, and buffered at 0.05 molar and pH 7 with phosphate solution. Infections were obtained from inocula that had been heated to 58° and 60° C., but not to 61° C. or higher temperatures.

Aging in vitro

Three experiments were performed by storing plant juice diluted 1 in 5 at 25° C., and inoculating at intervals to tobacco or *Nicotiana glutinosa*. In the first two experiments, the juice expressed from the diseased leaves was merely filtered through muslin. In the third, the inoculum was clarified and buffered with phosphate solution. Infections were obtained from inocula that had been kept for 10 or 11 days, but not for 16. In addition, virus was held at a few degrees above freezing point for more than a week without any noticeable diminution of infectivity.

IMMUNITY TESTS AGAINST VIRUS Y

A number of trials was made using tobacco and *Nicotiana glutinosa*, in which first virus Y and later the Brown's River virus were inoculated to the same plants. There were controls in all experiments inoculated singly with

virus Y and the Brown's River virus. The likeness of the symptoms produced by the two viruses, particularly on tobacco, rendered these experiments of relatively small value. However, where the strain of virus Y was sufficiently mild, there was an observable difference in the severity of symptoms produced by parallel inoculations on *Nicotiana glutinosa*. Using such a strain, some indications were obtained that it induced an immunity against subsequent infection with the Brown's River virus.

The most definite results were obtained with President potato systemically infected with virus Y. In some instances, full protection was afforded against the Brown's River virus. There was no necrotic reaction on inoculated leaves, whereas control President plants free from virus Y produced fully necrotic symptoms. However, some necrosis developed on the inoculated leaves of several other Y-infected plants after inoculation with the Brown's River virus. The necrosis was more diffuse than that usually produced at the site of inoculation on healthy plants, and may have been due in part to the original infection. There is no doubt that virus Y produced partial or complete immunity in President potato against subsequent infection with the Brown's River virus.

A few Y-infected plants of other varieties gave similar results when inoculated with the Brown's River virus.

DISCUSSION

The results of transmission tests and property studies leave little doubt that the Brown's River virus is virus C, and that virus C is a strain of virus Y (3, 4). The two instances of transmission by *Myzus persicae* suggest that virus C might occasionally be transmitted by this insect in the field. Several examples of field transmission under conditions of heavy aphid infestation (5) might be interpreted as rare examples of transmission by the normal vectors of virus Y.

Other examples of field transmission of what is possibly virus C are reported from New Zealand by Chamberlain (4). A virus complex carried in a potato variety, Aucklander Short-top, gave symptoms on a range of hosts that suggest it consisted of a mixture of virus X and B and another virus that causes top necrosis on Up-to-Date. In Chamberlain's greenhouse trials, no part of the virus complex was transmitted by Myzus persicae or Macrosiphum solani to healthy potatoes. Yet, "a severe top necrosis of several potato varieties was observed" in the field, and "the incidence of this disease was greatest in those lines which, in the previous season, had been grown in close proximity to the variety, Aucklander Short-top." Chamberlain's results are consistent with the view that the virus causing the top necrosis was virus C.

The chances of virus C causing serious economic losses are very small, except, possibly, in a new carrier variety that becomes infected as a seedling, or during the earliest stages of multiplication. From the incidence of the disease, it appears that this has been the mode of its perpetuation. Virus C

is seldom found except in carrier varieties, and, where it is found, it infects the whole or the greater portion of existing stocks. As an unusual variant of virus Y, it is of interest to students of virus diseases, but it is of no importance to the farmer.

SUMMARY

A virus isolated from an old Australian variety of potato is described, and identified with virus C. Its physical properties and host range, which are described, ally it closely with virus Y, but, except possibly in rare instances, the virus is not transmissible by the most efficient vector of virus Y, the aphid, Myzus persicae. The virus is unlikely to become widespread or produce serious losses in potato crops.

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH, DIVISION OF PLANT INDUSTRY, CANBERRA, A. C. T., AUSTRALIA.

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TOBACCO ANTHRACNOSE, A PLANT BED AND FIELD DISEASE1

E. A. WALKER² AND ELIZABETH WISE MCINROY³

(Accepted for publication February 15, 1945)

Tobacco anthracnose in Maryland was first observed in a commercial Medium Broadleaf tobacco plant bed on May 24, 1941, near Waldorf in Charles County. A leaf spot was found in plant beds treated for the control of tobacco blue mold with 1½ pounds of paradichlorobenzene to 100 square yards of plant bed per treatment. This conspicuous leaf spot was distinctly different from any injury previously experienced when tobacco plants were treated with this gas-producing substance. The spots were small, circular, and scattered over the leaf, resembling to some extent the injury produced when nitrate of soda granules are in contact with the leaves after top-dressing with this fertilizer. Plants in untreated plant beds adjacent to those receiving the paradichlorobenzene gas treatment also had leaf spot. These plant beds were established in "new land" and were surrounded by a wooded area to the north and west, and sloped eastward toward a weed strip used the previous season for tobacco plant beds.

Many tobacco plant beds were then visited in Charles, Prince Georges, and Calvert Counties, and this same leaf spot was found distributed quite generally in Southern Maryland. In several tobacco plant beds seedling blight was observed in small areas where many plants had died. These were surrounded by plants with leaf spots like those first observed in Charles County. A similar leaf spot was found in the College tobacco greenhouse where 64 strains of Maryland type tobacco were bedded. It was so destructive as a seedling blight by the first of June, that only a few live plants remained in some seed plots.

Symptoms on tobacco plants are distinct. Young spots are small, light green, slightly water-soaked, and considerably depressed. These pin-point depressions soon enlarge to form circular spots $\frac{1}{16}$ to $\frac{1}{8}$ inch in diameter. As the spots dry out they become papery thin and grayish-white, and are surrounded by a raised water-soaked border, which later becomes brownish. Larger spots may become zonated and have a darker center. Many small lateral veins are killed and turn brownish-black. This causes the affected leaf to become wrinkled and distorted, and a portion of it may die (Fig. 1, A). If spots are numerous there may be a general breakdown and death of the entire leaf. Small plants the size of a quarter or half dollar, when severely affected are stunted or killed, resulting in large barren spots in tobacco plant beds.

¹ Scientific Paper No. A76, Contribution No. 1931, Maryland Agricultural Experiment Station (Department of Botany).

² Formerly Pathologist, Emergency Plant Disease Prevention Project, Bureau Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture; now Assistant Professor of Plant Pathology, University of Maryland.

Plants large enough for transplanting had small cankers on the leaf midrib and petiole. These lesions are water-soaked at first, and later become sunken to form oblong greenish-brown lesions $\frac{1}{4}$ to $\frac{1}{2}$ inch long. Similar lesions, some circular and others oblong, were on the main stem (Fig. 1, B). These weaken the stem so that it breaks when plants are drawn for transplanting. Pink masses of spores surrounded by black tapering setae were in the lesions.

Isolations were made on 3 per cent malt agar, and a *Colletotrichum* was consistently isolated from plants with stem, petiole, and midrib lesions. The same organism was isolated from the leaf spots, which usually had few spores but numerous setae. A spore suspension was made and fresh tobacco seedlings were inoculated by spraying this ten-day-old, malt-agar spore suspen-

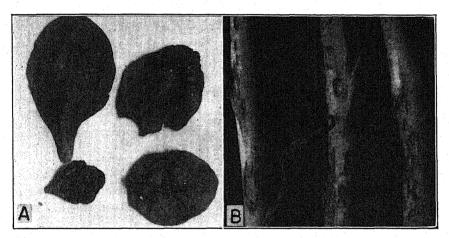


FIG. 1. Anthracnose on tobacco leaves and stems. A. Leaves of seedling tobacco plants with large anthracnose spots. The lower right leaf shows necrosis of the midrib and lateral veins. $\times \frac{1}{2}$ approx. B. Elongated anthracnose lesions on stems of tobacco seedlings. The stem to the right is practically girdled by the fungus. $\times 1$ approx.

sion on the leaves. After these plants had been in a moist dark chamber for 60 hours, the inoculated leaves were severely spotted, and some plants were killed. The *Colletotrichum* was recovered, and reinoculated onto other healthy tobacco plant leaves, on which anthracnose spots developed, and the same organism was again recovered. This was repeated again to establish its pathogenicity for tobacco. The tobacco plants used were of the Maryland Medium Broadleaf type generally grown in Maryland commercial plantings. Similar tests were made on numerous wild and cultivated host plants by the detached leaf and potted plant methods, and the fungus was pathogenic to pokeweed, geranium, begonia, tomato, and potato leaves.

Numerous isolations were made from tobacco anthracnose stem, petiole, and midrib lesions and leaf spots during the first season. *Colletotrichum* sp. was isolated from most of the diseased material, from which single spore or hyphal tip cultures were obtained for further laboratory cultural studies. *Gloeosporium* sp. was isolated directly from some tobacco anthracnose

lesions and other strains of *Gloeosporium* sp. were obtained from dark sectors in cultures of *Colletotrichum*. Sectoring was abundant on cultures of the *Colletotrichum*; and spiral growth was observed in two strains of *Colletotrichum* and in two of *Gloeosporium*.

Tobacco anthracnose was first recorded from commercial tobacco plant beds in the United States in 1941. Johnson and Valleau (6) observed a similar disease on tobacco plants in the Station beds in Kentucky in 1935, and they called the organism *Colletotrichum destructivum*. Tobacco anthracnose was first observed in Brazil by Averna-Sacca (1, pp. 221–223),

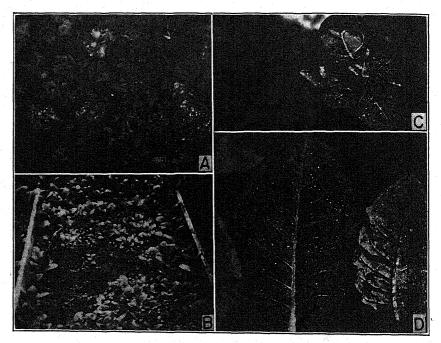


Fig. 2. Tobacco plants affected with anthracnose in plant beds, and infected leaves from plants grown in the field. A. Diseased plants with white circular leaf spots on lower leaves, and young upper leaves crinkled and distorted by anthracnose cankers on the midrib and lateral veins. B. Plant bed with plants killed by anthracnose while very small. Adjacent plants are small and dwarfed. C. Single plant from plant bed A showing leaf spots and crinkled bud leaves. $\times \frac{1}{5}$ approx. D. Middle leaves taken from field plants with small circular anthracnose spots. The leaf on the right has elongated young anthracnose cankers on the midrib. $\times \frac{1}{5}$ approx.

who studied the disease and named the causal organism Colletotrichum nicotianae. Böning (2, 3) in 1929 isolated and described the organism causing tobacco anthracnose in Germany as Colletotrichum tabacum. In 1932 Böning (4) isolated a similar organism, devoid of setae, to which the name Gloeosporium Desmaz. et Mont. was given. Tobacco anthracnose has been reported each year from Maryland since first observed in 1941 (9 through 15),* and from Pennsylvania in 1942 (5). The organisms obtained from

^{*}Type specimens have been deposited in the Mycological Collection of the Bureau of Plant Industry, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

Maryland tobacco anthracnose are being designated as Colletotrichum sp. and Gloeosporium sp.

During 1942 tobacco anthracnose became very severe in plant beds by May 18 in Prince Georges County (Fig. 2). A week later it was observed at one location near Gambrills, Anne Arundel County, where it had destroyed about 2,100 square yards of tobacco bed. The disease was very destructive in most plant beds visited in Southern Maryland. The plant beds at the Marlboro sub-station were infected, causing some loss from death of plants. Many diseased plants were transplanted to the field where the anthracnose disease continued to develop as leaf spots, leaf midrib and petiole cankers, and as stem cankers on the maturing plants. If the plants were set in very wet soil the disease developed rapidly on the new leaves, dwarfed the plants, and distorted the leaves. Cankers were also observed on the branches of the seed head and on the seed pods. In some fields anthracnose was observed on the "sucker" plants that developed after the crop was harvested. It is believed that the anthracnose organism is carried on the seed, and then carried to the fields on infected plants.

MARYLAND AGRICULTURAL EXPERIMENT STATION, COLLEGE PARK, MARYLAND.

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CURLY-TOP AND CALIFORNIA-ASTER-YELLOWS DISEASES OF FLAX

HENRY H. P. SEVERIN AND BYRON R. HOUSTON

(Accepted for publication February 15, 1945)

Flax, Linum usitatissimum L., was reported to be susceptible to the curly-top virus by Severin¹ in 1929 and the symptoms on experimentally infected plants were described. No naturally infected plants were found prior to March, 1944, at which time the disease was generally distributed throughout the flax-growing region of Central California, most of which is located in western Fresno County. The estimated infection varied from 0 in the late planted fields to 5 per cent in the early plantings.

RELATION BETWEEN PLANTING DATE AND INFECTION PERCENTAGES

There was a definite relation between time of planting and infection percentages. The fields planted in October and November, 1943, were more severely damaged than those planted at a later date. The beet leafhopper, Eutettix tenellus (Baker), is normally present in large numbers on native and introduced weeds in the floor of the valley during the summer and autumn months. Severin² has stated that the autumn dispersal from these weeds begins in October and continues through November. During this period in 1943 there was a shortage of rainfall, thus limiting the feeding areas of the leafhoppers to cultivated crops, the principal one of which was early planted flax. By the time the flax that had been planted after November had reached the seedling stage, most of the leafhoppers probably had flown to the overwintering regions in the foothills of the Inner Coast Range bounding the west side of the San Joaquin Valley. This normal autumn flight of the vector probably accounted for the failure of the disease to become established in the later plantings.

SYMPTOMS IN THE FIELD

The first symptom noticeable in small seedling flax is the production of irregularly shaped wavy leaves closely grouped at the growing point (Fig. 1). There is then a gradual yellowing of the entire plant followed by death in most cases. Plants 8 to 10 inches tall when infected often continue to grow and produce the described leaf symptoms and a coiling of the tip of the stem (Fig. 1). Subsequent branches from such plants are often bent away from their normal position resulting in a spreading rather than an upright growing habit.

The most striking symptoms are those in the flowers. Diseased plants which reach the flowering stage produce small and much shortened inflores-

¹ Severin, Henry H. P. Additional host plants of curly-top. Hilgardia 3: 595-629.

² Severin, Henry H. P. Field observations on the beet leafhopper, *Eutettix tenellus*, in California. Hilgardia 7: 281-350. 1933.

cences. The petals of the flowers emerge from the calyx but remain closely pressed together and do not expand and open as do the normal flowers (Fig. 2, A, B). The petals are thus held until dry at which time they fall away from the ovary in a group. The diseased petals are twisted and puckered by small blister-like elevations over their entire surface (Fig. 2, D). Little seed is produced by infected plants.

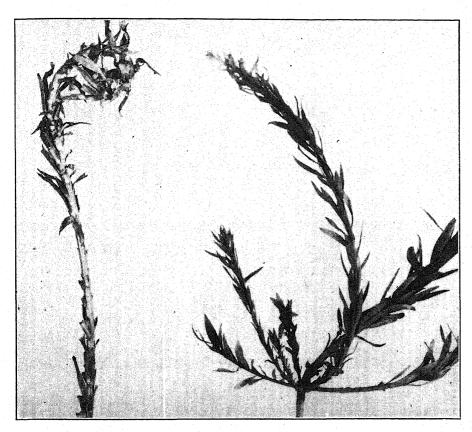


Fig. 1. Disease symptoms on flax plants naturally infected by the curly-top virus. The stem on the left has the distorted and coiled tip with closely clustered malformed leaves often found on infected plants. The plant on the right shows the spreading rather than normal upright branching habit. Distorted wavy leaves are near the top of the upper stem.

Nearly all infected plants have a marked orange-yellow discoloration in the region of the phloem in the tap root and crown.

RECOVERY OF VIRUS

The virus was recovered from naturally infected flax by previously non-infective beet leafhoppers and transferred to healthy sugar beets which developed typical symptoms of curly-top.

DISCUSSION

It is probable that with the present trend to earlier fall planting of flax there will be considerable curly-top infection in these fields. Leafhopper infestation in the fall of 1943 was such that no large losses resulted. However, early plantings adjacent to areas abounding with preferred food and breeding plants such as Russian thistle (Salsola kali L. var. tenuifolia G. F.

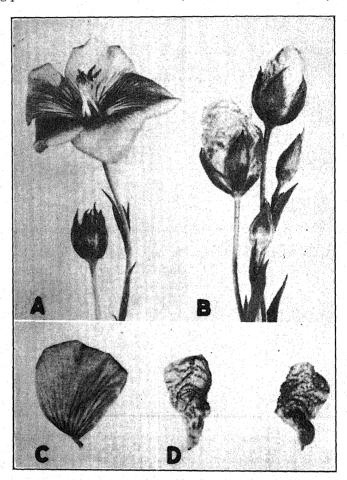


Fig. 2. Flax flower symptoms induced by the curly-top virus. A. Normal flax flower fully opened. B. Flowers same age as in A from diseased plant showing failure of bud to open. The entire inflorescence usually is in this condition. C. Petal from normal flower. D. Petals from diseased flower showing twisting and puckering in blister-like elevations.

W. Mey), bractscale (Atriplex bracteosa Wats.), and fogweed or silverscale (A. argentea subsp. expansa Wats.), which are host plants to both vector and virus, may easily suffer severe losses in years favorable to high leaf-hopper population.

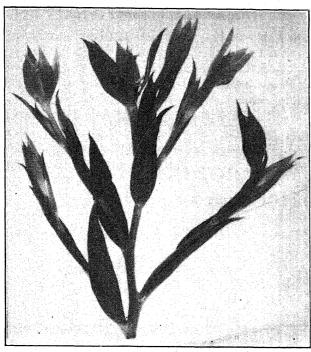


Fig. 3. Flax experimentally infected by the California aster-yellows virus showing secondary shoots arising from the axils of the leaves.

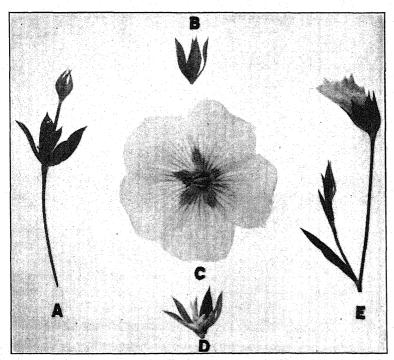


Fig. 4. Flax flower symptoms induced by the California-aster-yellows virus. A. Proliferation of flower. B. Flower with petals absent. C. Normal flower from control plant. D. Flower with reduced petals. E. Virescence or greening of flowers.

CALIFORNIA-ASTER-YELLOWS OF FLAX

Flax grown from seeds was experimentally infected with the California-aster-yellows virus by means of short-winged aster leafhopper, *Macrosteles divisus* (Uhl.), and the long-winged aster leafhopper, a race of the same species. The short-winged aster leafhopper failed to complete the nymphal stages, but a low population of long-winged adults was reared on flax.

The first symptom on experimentally infected flax is a yellowing of the apical leaves of the stems. Secondary shoots arise from the axils of the leaves (Fig. 3). Virescence or greening (Fig. 4, E) and proliferation of the flowers occur (Fig 4, A). The petals are often reduced (Fig. 4, C) or absent (Fig. 4, B).

Divisions of Entomology and Plant Pathology, University of California, Berkeley and Davis, California.

TRANSMISSION OF PEACH WART TO SWEET CHERRY!

S. M. ZELLER AND J. A. MILBRATH

(Accepted for publication February 15, 1945)

Peach wart has become known in the Pacific Northwest through the work of Blodgett² who first observed the disease in 1938. He reported the disease in eastern Oregon but it has now been found as far west as Yamhill County in this State.

Two Improved Elberta peach trees in Yamhill County, Oregon, with the wart disease have been the source of inoculum of the transmission studies reported here. The disease was transmitted by budding five trees of each of seven varieties of peaches, Early Crawford, Early Muir, J. H. Hale, Improved Elberta, Orange Cling, Rio Oso Gem, and Rochester. All inoculated trees bore warty fruits the following season. There were no leaf or stem symptoms, which would indicate that the source of inoculum was free of other viruses.

It has been our practice to index the stone fruit viruses on several hosts in the genus *Prunus*. In this connection the symptoms of peach wart on sweet cherry have proved of interest.

Five trees of the Napoleon (Royal Anne) variety and three trees each of the Lambert and Black Republican varieties were inoculated by budding. Trees inoculated in August begin to show symptoms by the next April and by May the symptoms are very characteristic in the new growth of the stems and in the newer leaves.

In the Napoleon variety the stem symptoms at first are limited to necrosis in the vascular ring. This necrosis as far as 12 to 15 inches back from the tips results in a general die-back (Fig. 1). Sometimes there is more than normal branching. In addition the internodes of the last few inches near the terminals of the several branches are shorter and considerably larger in diameter than normal. This condition and resulting "leafiness" of the terminals give a rosetted appearance. There is a tendency toward smaller, narrower leaves as a result of crowding in the rosettes.

The stem reaction in the Napoleon variety also occurs when the Black Republican and Lambert varieties of sweet cherry are inoculated with the peach-wart virus.

The mottling produced in the leaves of the Napoleon variety is more characteristic and distinct than that produced in Black Republican or Lambert varieties of cherry. The mottling seems to start with chlorosis bordering veinlets. This spreads out to form larger ring-like patterns or even somewhat rectangular bands or lines on either side of veins (Fig. 2). In May and June the symptoms are especially prominent on most of the

¹ Published as Technical Paper No. 450, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany. ² Blodgett, Earle C. Peach wart. Phytopath. 33: 21-32. 1943.

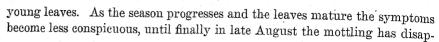




Fig. 1. Die-back of sweet cherry caused by peach-wart virus.

peared in all of the matured leaves, even though they may have been mottled earlier.

There is a similarity between the mottling produced in cherry leaves

by peach-wart virus and that produced by mild-mottle-leaf virus. Warty fruits, however, are not produced when peach trees are inoculated with mild mottle leaf of cherry. Cherry trees with the mild-mottle-leaf disease likewise do not have the stem symptoms described for peach wart in cherry.

The peach-wart virus was also transmitted from peach to cherry and back to peach without apparent attenuation. Buds from Napoleon and Black Republican cherry trees with symptoms of peach wart were used as inoculum in Early Crawford peach trees. Whether the virus came from peach through Napoleon cherry or through Black Republican cherry, the Early Crawford peach trees bore fruit with wart symptoms the next season.

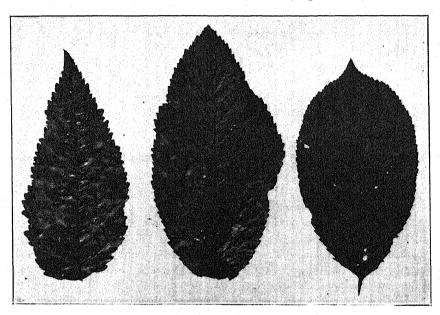


FIG. 2. Leaves of 3 stages of maturity from a Napoleon cherry tree inoculated with peach-wart virus. Young leaf with prominent mottling and two older leaves showing disappearance of mottling with maturity.

Cherry therefore may be a host of the peach-wart virus although this virus has not been found in cherry under natural conditions.

SUMMARY

Peach wart found in Improved Elberta trees in western Oregon was transmitted to seven varieties of peach trees and to Black Republican, Lambert, and Napoleon (Royal Anne) sweet cherry trees. The symptoms in sweet cherry leaves and stems are described. Peach-wart virus was also transmitted from the sweet cherry trees back to peach trees without apparent attenuation of the virus in cherry.

OREGON AGRICULTURAL EXPERIMENT STATION, CORVALLIS, OREGON.

ANATOMY OF BUCKSKIN-DISEASED PEACH AND CHERRY'

HENRY SCHNEIDER²

(Accepted for publication March 1, 1945)

INTRODUCTION

Anatomical changes induced by the presence of virus in plant tissues are of interest and importance for several reasons. They may serve to indicate the avenue of entry of virus into the plant and the subsequent path of migration. This may in turn be of assistance in determining the vector or vectors of a given virus. In many cases a knowledge of internal microscopic symptoms will lead to a better understanding of the external symptoms upon which diagnosis largely depends.

Necrosis of the sieve tubes is a characteristic anatomical change produced in buckskin-diseased peach on peach stock and in sweet cherry when on Mahaleb root stock. Rawlins and Thomas (16) noted that a disturbance occurred in the phloem of peach and cherry which was indicated by a woundgum reaction when sections were treated with phloroglucinol and hydrochloric acid. They later stated that the phloem necrosis resembled that of potato leaf roll (21). Necrotic spots frequently form in the leaves of affected peach trees. The histology and histochemistry of the formation of these spots has been briefly described (16). The present paper considers the structure and development of the phloem of diseased peach and cherry and compares this tissue with the phloem of the healthy peach and cherry as described in a previous paper (17). Studies on the histochemistry of affected phloem tissues and on the anatomy of peach and cherry on incompatible root stock are also described.

EXTERNAL SYMPTOMS OF THE DISEASE IN PEACH AND CHERRY

Buckskin disease was first described and so named on the basis of material of sweet cherry (*Prunus avium* L.) collected at Green Valley, Solano County, California (14). The principal symptoms were failure of fruits to mature and the development of a leathery fruit surface. Leaves of infected cherry trees often became bright orange to red in the basal part of the lamina. Some years later a disease of peach at first designated as "leaf casting yellows" was also found to be caused by the buckskin virus (20). The chief symptoms in peach (*P. Persica* Sieb. and Zucc.) are yellowing and rolling of leaves, death and abscission of irregular areas in the lamina, and shedding of fruit and leaves of affected branches in early summer. Other symptoms

¹ Part of a thesis submitted in partial fulfillment of requirements for the degree of Dector of Philosophy, University of California, 1943.

² The author is indebted to Drs. Katherine Esau and H. Earl Thomas for guidance in the work and preparation of the manuscript. Dr. Thomas and T. E. Rawlins supplied the plant material used. Several members of the Division of Plant Pathology of the University of California and the Guayule Research Project kindly read and criticized the manuscript.

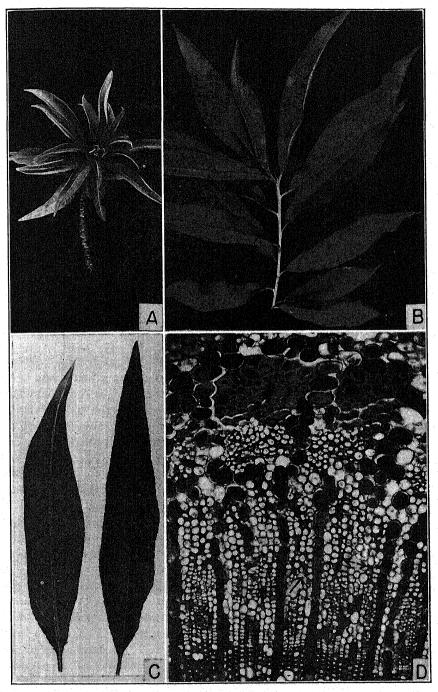


Fig. 1. A. Phillips Cling peach stem affected by the Palo Alto strain of the buckskin virus (\times 0.4). B. A healthy peach shoot (\times 0.4). C. Left. Swollen veins in a Muir seedling leaf affected by the Palo Alto strain of buckskin virus. Right. A normal leaf (\times 1.1). D. Transverse section of noninfected Palora peach stem (on Myrobalan stock) $1\frac{1}{2}$ inches from the stem tip. Arrows point to three of the necrotic sieve tubes. In the new band of secondary phloem the sieve tubes have not undergone turgor expansion (\times 200).

produced by two distinct strains of buckskin virus on cherry and peach have been described in some detail (21).

Two new strains of buckskin virus³ were used in some of the tests. One was collected in peach at Palo Alto, California. It has not been found in sweet cherry nor has it been successfully inoculated into a sufficient number of cherries to permit a detailed study of symptoms in them. The early symptoms produced by this strain in peach are similar to those produced by the Green Valley virus. These are followed, however, by extreme dwarfing of leaves and shortening of internodes (Fig. 1, A and B) while the terminal buds continue to grow slowly far beyond the normal growth period. Another strain of buckskin virus obtained from a peach orchard at Merced, California, produced symptoms similar to those caused by the strain of virus found at Green Valley, but the Phillips Cling variety of peach was more severely affected by it.

METHODS AND MATERIALS

The methods used on the diseased material are the same as those described elsewhere for healthy trees (17). Unless otherwise specified it is understood that the trees were inoculated with the Green Valley strain of virus. Special methods are given later for the work on diseased cherry.

ANATOMY OF DISEASED PEACH

Pathological Anatomy of the Primary and Early Secondary Phloem

In general the pathological changes observed in the primary and early secondary phloem of diseased peach trees are clear cut. At the onset of the disease the sieve tubes and companion cells become necrotic and collapse in the older part of the phloem (Fig. 2 and 3, B). In these early stages, the necrosis may then proceed even to the youngest sieve tubes (Fig. 4, B). Following this, new phloem may be formed to replace the affected phloem, and the sieve tubes in this new tissue may also become necrotic (Fig. 5, B, and 8, D). Although these are the usual features of the diseased phloem tissue, other characteristics have been noted in diseased stems and are described later with reference to the conditions under which they occurred. The structure of the median trace of leaf 13 from the shoot tip at a point just below where it diverges from the stem and above where it was first branched is used for comparison with healthy trees as described elsewhere (17).

In the greenhouse, young normal appearing shoots growing from the upper portion of diseased trees that had been cut back had no phloem necrosis or other abnormalities. In some cases such tips were found to be free of virus when they were grafted to healthy trees. Three weeks later symptoms were present on leaves two or three inches from the tips of the new stems on the trees that had been cut back. Sieve tubes of the primary phloem had become necrotic in the petioles of leaves with symptoms and a wide band of secondary phloem had been produced. In comparable normal petioles little secondary phloem is present and the metaphloem is not obliter-

³ These strains were collected and tested and are here described by H. Earl Thomas.

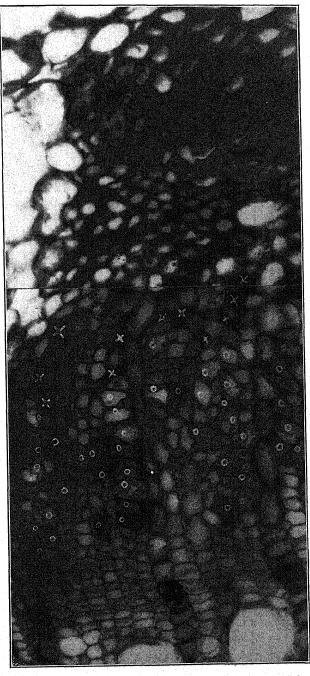


FIG. 2. Transverse section of a young peach stem, newly affected by Green Valley buckskin virus. Some of the older sieve tubes of the primary phloem were obliterated and others were necrotic. The older sieve tubes in the secondary phloem were becoming necrotic (indicated by x's). Normal sieve tubes are marked with circles. The outer sieve tubes that have not collapsed do not have thick nacré walls (x 575).

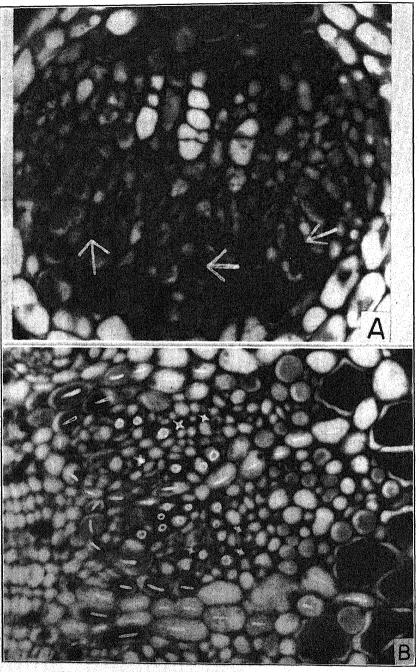


Fig. 3. Transverse sections from peach shoots affected by Green Valley buckskin virus. A. The median bundle of leaf base 7. Arrows point to necrotic protophloem sieve tubes (×1580). B. Phloem strands of the median trace from leaf 13 two mm. from the stem tip. The trace was not so profoundly affected as other traces at the same level. Several of the older sieve tubes are semi-collapsed (marked with ×'s). Normal sieve tubes are marked with circles. Dashes indicate the primary ray cells and the parenchyma cells between the primary phloem and the procambium (×590).

ated, even in old leaves. Necrosis of the metaphloem and older secondary sieve tubes in the stem below the affected leaves had also occurred (Fig. 2). Normally no secondary sieve tubes would be obliterated at this stage and some of the metaphloem sieve tubes would still be functioning. Sieve-tube necrosis was absent or just appearing in parts of the stem above the affected leaves.

Shoots from a tree affected by the virus for several weeks had ceased elongating and symptoms were present in leaves about five inches from the tips of the stems. Necrosis had occurred in many of the sieve tubes beginning with those first formed in the leaf primordia and stem (Fig. 3, A) and continuing with those derived from the cambium in parts of the stem producing secondary growth. The outer primary phloem fiber initials that had undergone turgor expansion but had not yet developed secondary walls, were occasionally collapsed in the leaf traces. The cambium in some places contained heavily staining precipitates. Its orderly arrangement was disturbed and cells were frequently crushed. In the median trace from leaf 13 of another of the shoots affected, for several weeks only a few more sieve tubes had collapsed under the influence of necrosis than would collapse in normal obliteration (Fig. 3, B). In some of the younger traces at the same level, however, all the sieve tubes had collapsed. Apparently the virus is not present in equal quantities or is not equally active in all leaves and traces. This is substantiated by the general experience that not all buds from affected shoots transmit the virus when transferred to healthy plants.

Another stem had severe leaf symptoms at its tip and appeared to have stopped growing as a result of the disease, but sections showed that a number of leaf primordia had been produced since the onset of the disease. Apparently all the sieve tubes in the stem at the time of the onset of the disease as well as some of the partially differentiated fibers were killed (Fig. 4, A and B). New imperfectly differentiated sieve tubes were present at the time the material was taken for sectioning. Parenchyma cells apparently produced after the onset of the disease had thin walls, wavy in outline. In the primary phloem of newly initiated traces, there was no differentiation of primary ray cells or of large fiber initials. A few recently formed phloem mother cells had divided to form sieve tubes and companion cells, but the sieve tubes did not undergo turgor expansion (Fig. 4, A). In tissues in and around the median trace of leaf 21, most of which was probably initiated before becoming affected by the virus, there were necrotic cells other than sieve tubes (Fig. 4, B). Some of the parenchyma cells were hypertrophied. All of the cells of the outer row of primary phloem fiber initials were collapsed. This type of flattening of the outer fiber cell initials was found in all traces at this level.

Some diseased shoots continue to grow after infection of branches (Fig. 1, A). The internodes of the new growth, however, do not elongate fully and the leaves are small and rolled. The veins soon become swollen (Fig. 1, C). The development of the phloem was somewhat abnormal in several such

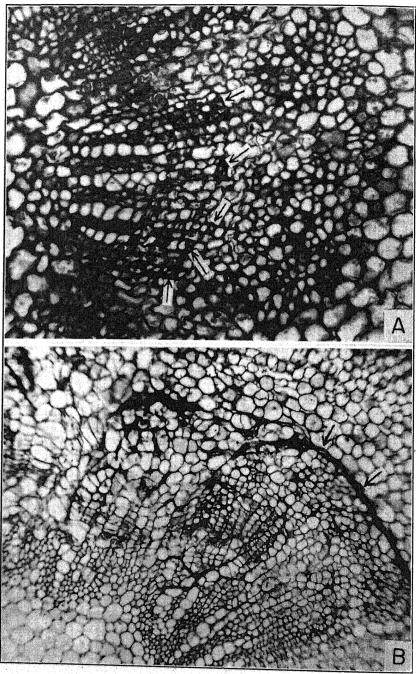


Fig. 4. Transverse sections of a peach stem affected by Green Valley buckskin virus. Some growth was produced after the onset of the disease. A. Median trace from the 13th leaf 0.8 mm, below the stem tip. Fiber initials failed to enlarge and the primary phloem did not form elliptical strands. The sieve tubes marked by arrows in the portion of the phloem produced most recently have not undergone turgor expansion after division of the phloem mother cells (×460). B. Median trace from the 21st leaf 11 mm, from the stem tip. Another branch trace appears to its left. Necrosis had occurred in the outer row of fiber initials (indicated by arrows), in the cortical parenchyma cells, sieve tubes, phloem, parenchyma cells, cambium, and xylem parenchyma cells. Some hypertrophied cells are present (×180).

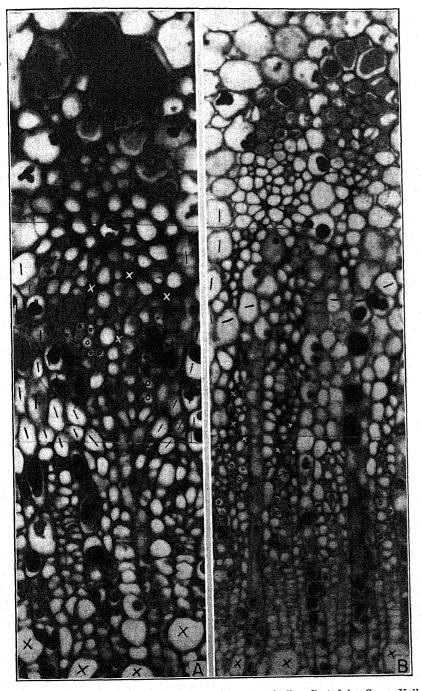


Fig. 5. Transverse sections of a peach stem chronically affected by Green Valley buckskin virus. A. Median trace from leaf 13 within the stem. Metaphloem sieve tubes do not have nacré walls. No secondary sieve tubes have differentiated in the secondary phloem. Otherwise the strand is normal. Details are: dashes, primary ray cells; x's, obliterated sieve tubes; circles, sieve tubes; x's in xylem are vessels (x 570). B. A branch trace five mm. from the stem tip. Abnormal amounts of secondary phloem were produced and then obliterated abnormally soon. The arrow points to callus on a sieve plate. Dashes indicate the approximate boundary between the primary and secondary phloem; x's indicate necrotic sieve tubes bordering the region of normal ones; circles indicate sieve tubes; x's in the xylem indicate vessels (x 400).

shoots. In one such shoot affected by the Green Valley strain of virus the youngest leaf primordia to contain sieve tubes developed apparently normally, but there was some indication that the older sieve tubes became necrotic sooner than they would have normally. The median trace of leaf 13 had developed very much like that of a normal stem: the primary phloem was laid down in elliptical strands that were more or less isolated from the secondary phloem by parenchyma cells; the fiber initials expanded normally; and about the usual number of sieve tubes were in the primary phloem (Fig. 5, A). It differed from the 13th trace of an elongating stem or one ceasing to elongate in that it was producing secondary phloem. However, in this

TABLE 1.—Relative age of the leaf primordium with respect to differentiation of the vascular elements and necrosis of sieve tubes

	Stem tip number, nature of growth, and symptoms	Number of the leaf primordium in which character or change first appeared			
			Leaf base		Petiole
		Sieve tubes	Thick- walled xylem	Phloem obliteration or necrosis	Thick- walled xylem
	Succulent, with apical growth. No symptoms Apical growth nearly stopped. Symptoms on 5th expanded leaf. Newly	5	7	11	6
3.	infected Apical growth stopping. Symptoms on 8th expanded leaf. Infection 7 weeks old		8	11 6a	
4. 5.	Same as 3	5	7 6	7b 6c	6
6.	Nodes short and leaves small. Chronic infection		7	7ª	7

^a Leaf bases 7 and 8 contained partially collapsed sieve tubes and leaf base 9 showed complete collapse of 5 out of 7 of them in some sections. (The degree to which a section of a sieve tube is collapsed depends on its proximity to a sieve plate.)

b Two sieve tubes crushed and one flattened (Fig. 3, A). c All of the sieve tubes were collapsed in trace 7.

d Four out of five sieve tubes were crushed in trace 8.

respect it resembled a normal trace in a stem that had not produced apical growth for several weeks, except that no mature sieve tubes were in the secondary phloem of the diseased trace. Normally such maturation proceeds before so many cells are initiated. Nacré walls (thick crenulated walls with a pearly luster) were absent in the sieve tubes of the metaphloem. Fiber initials had not started to lay down secondary walls as occurred in the 13th median leaf trace of healthy Orange Cling peach stems which were growing slowly. At lower levels in the stem many more sieve tubes were necrotic than are normally obliterated (Fig. 5, B).

In summary it may be said that the most common anatomical change caused by the disease was the necrosis of sieve tubes. This necrosis was

similar to obliteration but occurred in sieve tubes before their normal period of functioning was completed. In severe cases the youngest sieve tubes became necrotic (Table 1), the orderly arrangement of cells in the cambium was affected, some parenchyma cells of the phloem and cortex collapsed while others were hypertrophied, and fiber initials in some cases were collapsed. Ontogeny after such severe attacks was abnormal. Primary rays did not develop nor did sieve tubes and fiber initials undergo turgor expansion. In stems that continued slow growth with shortened internodes and swollen leaf veins after arrival of the virus at the stem tip, ontogeny approached the normal; but the older sieve tubes became necrotic somewhat before they should normally have been obliterated and then they were replaced by new phloem.

Histochemical Changes in the Secondary Phloem

It has been reported that wound gum was present in the secondary phloem of peach affected by buckskin virus (16). According to Küster (11), wound gum is characterized by the following properties. It does not dissolve or swell in water, it is insoluble in alcohol, ether, carbondisulphide, potassium hydroxide, sulphuric acid, cold nitric acid, and cold aqua regia. It is soluble in warm nitric acid (concentration not given) and in a mixture of HCl and KClO₃. It stains red with phloroglucinol and HCl. Hewitt (9) in his studies on olive regarded wound gum as a water-insoluble substance which turns red when treated with phloroglucinol and hydrochloric acid but gives a negative reaction with the Maule test for lignin. The material here referred to as wound gum does not have all of the properties ascribed to it by Küster, but it does have the properties of the substance in olive abscission layers which Hewitt refers to as wound gum.

Wound gum was confined for the most part to the walls and contents of collapsed sieve tubes of diseased peach, but in some instances it extended into the middle lamella of adjacent parenchyma cells. Sieve tubes first lose their thick nacré walls and callus appears on the sieve plates. Later they collapse and wound gum may be observed. Sieve tubes may be completely flattened by the pressure of adjacent cells (Fig. 6, B and D). It was noted at times that not all of the necrotic sieve tubes contained wound gum; although, the time for its synthesis in the process of necrosis may have been passed. Wound gum was not present in the primary sieve tubes of peach.

The phloroglucinol-staining material in the walls of buckskin-diseased sieve tubes was not removed after sections cut in paraffin were treated in 10 per cent nitric acid in a test tube immersed in a bath of boiling water for three hours. However, it no longer reacted with phloroglucinol and HCl after this treatment. Wound gum was not completely removed from apple wood affected by *Polystictus versicolor* when subjected to the same treatment. It was not removed from the diseased sieve tubes of peach or from vessels of diseased apple wood after treatment in a mixture of equal parts of saturated KClO₃ and 18 per cent HCl for 18 hours. This mixture

yields chlorine gas as an end product. Sieve-tube walls of peach remain yellow when tests are made for cellulose with IKI and $\rm H_2SO_4$ after these treatments. After removal of the wound gum by alternate treatments with

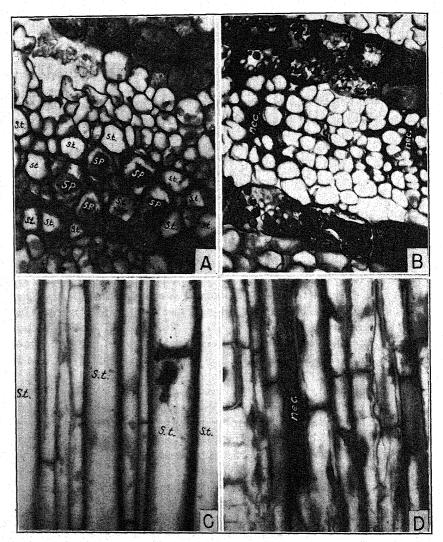


FIG. 6. Sections of secondary phloem of peach taken in December from greenhouse trees. A. Transverse section of healthy phloem between two rays. Sieve tubes have thick walls and sieve plates are in view in some of them. Calcium oxalate crystals have caused several parenchyma cell walls to disintegrate. B. Transverse section of buckskin-diseased phloem. All sieve tubes and companion cells have become necrotic. C. Longitudinal radial section of healthy phloem. One sieve plate is in view. D. Longitudinal radial section of buckskin-diseased phloem. One sieve tube and companion cell have degenerated. (A and B, × 465; C and D, × 640. s.p.—sieve plate; s.t.—sieve tube; nec.—necrosis.)

chlorine water and 2 per cent Na₂SO₃ at 95° C. (the method of Cross and Bevan for removing lignin) the affected walls stained much darker blue than

adjacent parenchyma cells when tested for cellulose with IKI and H₂SO₄. Occasionally weak coloration was obtained in affected sieve tubes when sections were treated by the method of Maule for lignin.

Stem Cankers

When a virulent strain of virus or a very susceptible peach variety is involved, rapidly growing shoots frequently develop cankers on the stem soon after the onset of disease. In Phillips Cling peaches affected by a strain of virus found in an orchard at Merced, California, this was particularly obvious. In the cortex of one such stem there was a large gum pocket and the cells around it from the epidermis to the xylem were necrotic (Fig. 7, A). Two smaller gum pockets were in the phloem and cambial region of the necrotic tissue. In the living tissue near the canker various abnormalities occurred. In the unlignified area of the xylem just below the cambium, gum ducts were forming. The cambium was abnormal in that it was not a layer of periclinally dividing rectangular cells. Instead, cells had divided in all directions and had become polygonal. In the most recently produced phloem the sieve tubes had not matured. In the older secondary phloem and in the primary phloem, the sieve tubes were necrotic. In another canker, a portion of the cortex and phloem were isolated by a periderm (Fig. 7, B). Primary phloem fiber strands were also isolated by periderm layers. Gum ducts were in the unlignified xylem tissue just beneath the cambium. In diseased material of a naturally infected seedling peach collected at Palo Alto, California, isolation of portions of the cortex and of the primary phloem fibers by periderms was very common. Gum ducts in the wood were frequently observed.

Anatomy of Swollen Veins in Peach Leaves Affected by the Buckskin Virus

Most leaves on peach branches affected by buckskin are prematurely abscissed. The veins of the older leaves that do not fall often become greatly enlarged when the plants are grown under glass (Fig. 1, C). Vein swelling is also seen occasionally in affected orchard trees. Rawlins and Thomas (16) reported that the swelling results from hypertrophy and hyperplasia in the phloem area; and that because of the vein-swelling the upper epidermis and palisade tissues separate from the lower epidermis and spongy parenchyma. Cavities arise on both sides of the vein. Cells of the bundle sheath frequently expand into the cavities and become quite large. The writers observed no necrosis.

In the present work swollen veins were characterized by necrotic sieve tubes and an abnormal production of secondary phloem. Occasionally secondary xylem has been observed in sections of swollen veins. Most of the phloem cells produced were parenchyma cells, rectangular when observed in a radial section. The sieve tubes were crushed and could be identified by the callus, stained with lacmoid, on their end walls.

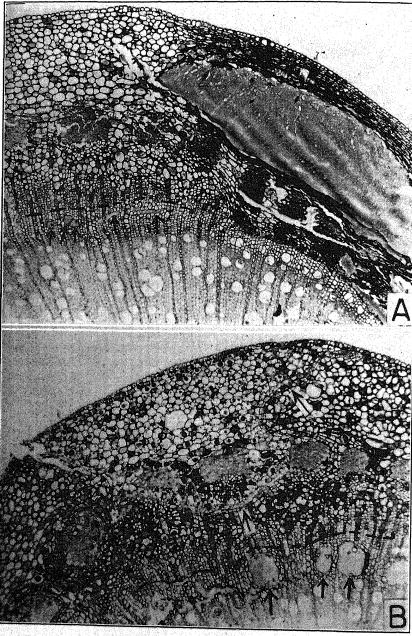


Fig. 7. Transverse sections of cankers in Phillips Cling peach stem affected by a strain of buckskin virus found at Merced, California. The position of the cambium is indicated by dashes. A. The canker on the right contains gum pockets and dead collapsed cells of tissues from the epidermis to the xylem. To the left of the canker, necrotic sieve tubes appear in the metaphloem and outer secondary phloem. There are gum pockets in the unlignified xylem (indicated by arrows). B. Another canker in which the dead area of the cortex and phloem is walled off by a periderm (indicated by arrows). A strand of primary phloem fibers to the left is also isolated by a periderm. Gum ducts in the xylem are indicated by arrows. Dashes mark the position of the vascular cambium. (Both × 95.)

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Affected petioles show a similar anatomical picture (Fig. 8). All of the primary phloem sieve tubes may become necrotic as well as the older abnormally produced secondary sieve tubes. Longitudinal sections of the abnormally produced secondary phloem show rectangular parenchyma cells with crushed sieve tubes between them. The presence of sieve plates in the end walls of the crushed sieve tubes has not been ascertained, but staining with lacmoid indicated that callus had developed.

Swollen Veins and Phloem Degeneration Caused by Ringing of Stems of Disease-Free Peach Trees

Leaves above an injured portion of stem may develop swollen veins. This suggested an experiment to determine if ringing would cause vein swelling similar to that in buckskin-diseased trees. A ring of bark about one-half inch wide was removed from each of several branches. The ring was wrapped with grafting tape to prevent drying. In an Orange Cling peach tree girdled in July the leaves eventually turned yellow, developed swollen veins, and many abscissed. Leaves of nonringed branches remained green, with normal veins. Sections of veins and petioles of leaves from ringed branches indicated that the cambium produced excessive amounts of secondary phloem. The newly produced sieve tubes resembled normal ones, and the original primary sieve tubes developed callus and were obliterated. Occasionally veins are very profoundly affected. In one such case cells of the xylem and phloem on one side of the vein became hypertrophied. The cambial region was very active and produced an abundance of flattened cells. Normally the metaphloem functions during the life of the leaf, with production of a trace of secondary phloem in petioles but none in lateral veins.

Two weeks after having been ringed the peach stems showed considerable change in the phloem in sections taken one-half inch above and below the ring. Leaves above the ring were still green and normal at this time. In the sections one-half inch below the ring vertical gum pockets had formed in the unlignified xylem mother cells just beneath the cambium. The cambium was not active and the sieve tubes still had thick nacré walls. No wound gum was present. Above the ring the sieve tubes showed a wound gum reaction of medium intensity, gum pockets had formed in the outer xylem, the cambium was very actively producing new xylem and phloem, and the older secondary sieve tubes were partially collapsed. Callus was observed in some of the sieve plates of the collapsed sieve tubes. Sections three and one-half inches below the ring were normal. In sections taken four inches above the ring the sieve tubes in the outer half of the secondary phloem were partially collapsed and their sieve plates were callused, but the nacré walls were still present. Phloem in sections thirteen inches above the ring were normal. Two nonringed branches were used as checks. Stem sections from immediately above the rings of branches ringed three months earlier revealed that the sieve tubes in about the outer three quarters of the phloem had lost their nacré walls and were collapsed. The functioning sieve

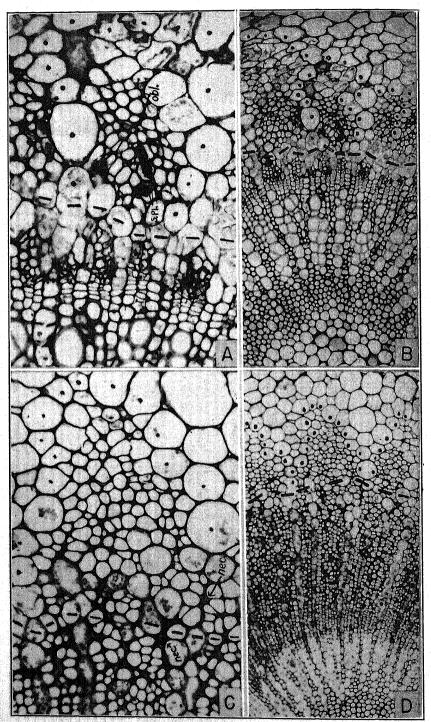


Fig. 8. Cross sections of peach leaf petioles. A and B healthy. (B lower magnification of A for orientation.) Dashes set off the primary phloem strands from the secon-

tubes in the quarter of the phloem near the cambium were small but otherwise appeared to be normal.

Phloem Degeneration in Healthy and Buckskin-Diseased Peach on Incompatible Rootstocks

Myrobalan (Prunus cerasifera Ehrh.) rootstocks are not completely compatible with peach tops. After peach scions or buds on such stocks grow to be several feet long under lath or in the greenhouse the leaves often roll, turn yellow, have swollen veins, and drop prematurely. Since these symptoms are similar to those of a buckskin-diseased peach, except that no death and abscission of irregular areas of the leaves occurs, the anatomy of such shoots is of interest. In several such stems a positive test for wound gum was obtained in the phloem. In a stained section of one such stem the sieve tubes of the metaphloem and the outer half of the secondary phloem were necrotic (Fig. 1, D). The inner part of the secondary phloem consisted of new tissue in which the sieve tubes were about half the size of the average parenchyma cells. The sieve tubes evidently had failed to enlarge after the division of the phloem mother cells.

No wound gum was observed immediately below buckskin-diseased peach scions (Merced and Palo Alto strains) in four Myrobalan trees or in the trunk or tap root of one of them. The peach scions had severe phloem injury.

ANATOMY OF BUCKSKIN-DISEASED CHERRY

Rawlins and Thomas (21) reported that wound gum is present in the phloem of stems of young buckskin-diseased sweet cherry tops on Mahaleb stock. When tops on Mahaleb stock become infected they die; however, such infections rarely occur naturally. The vegetative growth of trees on Mazzard roots on the other hand is not seriously affected by the disease (15). Examination of the phloem of these plants has suggested some explanation of these phenomena.

Methods and Materials

Necrosis of sieve tubes caused by buckskin virus in cherry was determined in many instances by a positive test for wound gum in the sieve tubes. Sliding microtome sections were used. The coloring by phloroglucinol and HCl due to the presence of wound gum was graded into three classes depending on its intensity and the area it covered. The location of the gum was

dary phloem of which only a trace is formed. The inner side of the primary phloem strands contains functioning metaphloem sieve tubes. The outer portion is composed of protophloem cells which enlarged and lengthened as the protophloem sieve tubes were obliterated. In stems, such cells have secondary walls and are the primary phloem fibers, spl.—sieve plates. obl.—obliterated cells. Dots in parenchyma cells adjoining primary phloem strands indicate the approximate division between the cortex and primary phloem. C. Buckskin-diseased petiole. The metaphloem sieve tubes are necrotic (outer margin of the metaphloem is indicated by dots in adjoining parenchyma cells). There are no large parenchyma cells separating the primary phloem from the secondary phloem. D. Lower magnification of C showing a wide band of secondary phloem which was produced and in which the sieve tubes became necrotic. Dashes indicate the approximate division between the primary and secondary phloem. nec. = necrosis. (A and C, × 330; B and D, × 125.)

also recorded in some cases. Lacmoid stain was used at times to determine the presence of definitive callus on the sieve plates. The presence of callus in amounts sufficient to completely fill the pores in the sieve plates was regarded as the first sign of cessation of functioning of the sieve tubes. Sections were made at several places on each tree. Celloidin and paraffin sections were also prepared from bark of several of the trees from an experimental field plot.

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Wound Gum in Sieve Tubes of Healthy Cherry Trees with Mahaleb Root Stocks

To determine whether healthy trees contained wound gum the phloem of four young trees grown in the greenhouse was studied during the summer. A band of large sieve tubes formed during the spring and summer is here called the summer phloem, and the narrow band of smaller sieve tubes produced in the autumn is called winter phloem (17). Occasional sieve tubes of the old nonfunctioning phloem and outer summer phloem in a side branch of one tree, and a few sieve tubes of the main trunk of another contained wound gum. Sections through the main trunk above and below the bud union of one tree and through the tap root of another tree revealed no wound gum.

In addition, three noninoculated Napoleon cherry trees on Mahaleb stock which were dying from root rot were also sectioned through the trunk. One was free of wound gum and the other two contained occasional sieve tubes with gum in the outer border of the summer phloem and in the previous winter's phloem.

The trunk and older limbs of healthy Napoleon orchard trees with Mahaleb root stock showed wound gum more often than trees grown in the greenhouse. Most of the wound gum was formed in the autumn. At this time definitive callus formed on the sieve plates somewhat earlier in the Mahaleb stock than in the sweet-cherry tops. Such callus formation began in the older sieve tubes. This difference in callus formation was evident in some sections made about an inch above and below the graft unions of four Napoleon cherry scions on Mahaleb cherry stock. The branches were from four to six inches in diameter and were still in full leaf. The cessation of sieve-tube function in the outer half of the Mahaleb phloem probably caused a barrier to translocation in the outer half of the still active phloem of the Napoleon tops and played a part in the necrosis of sieve tubes in the Napoleon tops. There were usually more sieve tubes with wound gum above the union than below. The fact that the width of the summer phloem of Mahaleb stock in five orchard trees was only about five-sevenths of that of the Napoleon top is also of interest in this connection. Likewise the Mahaleb stock of a greenhouse tree had a band of summer sieve tubes 175 microns wide as compared to 290 microns for the Napoleon top in sections taken above and below the bud union. The ratio of parenchyma cells to sieve tubes in the two species appeared to be comparable but no detailed study

was made. In side branches of sweet cherry tops taken from the same orchard trees several feet from the unions there was little or no gumming of sieve tubes.

In sections from orchard collections made in the winter, three of ten side branches near the bases of trees gave mild wound-gum reactions, five contained occasional sieve tubes with wound gum, and one was free of wound gum. The mild reactions were observed 400 to 500 microns from the cambium.

In the summer, sieve tubes with wound gum were found in the phloem of the previous winter and early spring. Sections above the bud union from a healthy tree budded near the ground level contained a few sieve tubes with wound gum on the outer margin of the summer phloem, but no gum was present in a branch near the top of the tree. Two trees which were dying because of girdling by gophers did not contain gum in the summer phloem, but a small amount was in the previous winter's phloem. One section made above a bacterial gummosis canker on a large limb showed a mild reaction in the outer part of the summer phloem and a strong reaction occurred in the previous summer's phloem in the Mahaleb stock below the canker.

These observations show that wound gum is sometimes found in non-functioning winter phloem and in the outer summer phloem of apparently healthy orchard trees on Mahaleb stock. Apparently sweet cherry tops are not entirely compatible with Mahaleb stock. The slight incompatibility between the two cherry species appears to be caused by differences in phloem area and in differences in the time of callus formation in the autumn.

Wound Gum in Sieve Tubes of Buckskin-Diseased Trees on Mahaleb Stocks

Four Napoleon trees on Mahaleb rootstock, growing in a glasshouse and inoculated with Green Valley buckskin virus, were sectioned in different places in each tree just as symptoms were beginning to show. The results are illustrated in figure 9 which gives a diagrammatic longitudinal view of the phloem of one of the trees and is characteristic of all of them. The phloem with no wound gum in the Napoleon tops is of about the same width as the summer phloem of healthy trees, but only about 70 microns of unaffected phloem remains in the Mahaleb stock just below the bud union. The 70 microns include the cambium and partially differentiated sieve tubes. Only a few spots of wound gum were present ten inches below the union and no gum at all was present below a branch of the Mahaleb stock. The side branch of the Mahaleb stock did not contain wound gum. Seven branch roots at distances of eight to twelve inches from the bud unions of two of the other trees similarly affected at the graft unions either contained no gum or only occasional spots in the outer part of the phloem. The fourth tree gave a reaction of medium intensity in the only root sectioned. A tree which had lost all of its leaves because of the disease had wound gum in the sieve tubes of the phloem much nearer to the cambium in the Napoleon top than had been the case in the trees just beginning to show symptoms.

Tests have been made for wound gum in diseased orchard trees. Measurements of the phloem in four diseased Napoleon scions on forked Mahaleb stocks in the fall indicated that the region near the union contained two and one-half times the amount of summer phloem as did healthy Napoleon branches on the other fork of the same Mahaleb trees. The abnormal tissue contained more phloem parenchyma cells and fewer sieve tubes than normal summer phloem, and the sieve tubes in the older part were necrotic. The sieve tubes of the Mahaleb stock were necrotic nearer the cambium than were those of the Napoleon tops, and in two cases they were ultimately killed all the way to the cambium. Material collected at other times of the year has also

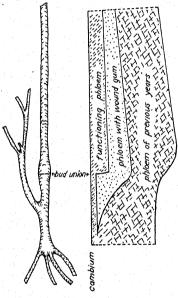


Fig. 9. Left. Sketch of a cherry tree on a Mahaleb cherry stock. The larger branch which was severely affected by the buckskin virus is the Napoleon cherry portion. The smaller Mahaleb branch did not contain wound gum in the phloem. Right. A diagram of the phloem of the right side of the tree showing the location of wound gum. Sieve tubes in most of the mature phloem of the Mahaleb had become necrotic.

shown that more phloem tissue is killed in the Mahaleb stock than in the Napoleon tops (Fig. 10).

Studies were made of the occurrence of wound gum at levels below unions of diseased orchard trees on Mahaleb stock. Injury was just as severe in portions of the stem nine to twelve inches below the graft union as immediately below the union in four branches of Mahaleb with severely affected Napoleon scions. In the case of a tree budded near the ground level the amount of injury decreased at increased distances below the union. The tree was just beginning to show leaf symptoms. At 38 inches below the union only occasional sieve tubes contained wound gum. Another tree, inoculated at the same time but taken six weeks later when the leaves were wilted and yellow, had uniform amounts of wound gum as far as twenty-two inches below the bud union.

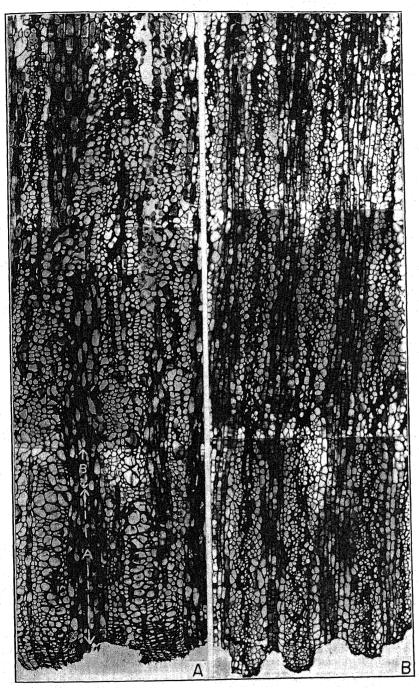


Fig. 10. Transverse sections from a buckskin-diseased Napoleon cherry tree on Mahaleb rootstock taken above and below the bud union. The tree was nine years old and was budded near the ground. It was inoculated in February, 1940, and the leaves were wilting and turning yellow in September, 1941, when the sections were made. A. Napoleon top. The width of phloem (A) between the arrows (320 μ) is composed of functioning phloem. The width (B) (180 μ) contains callused sieve tubes and the remainder of the phloem contains callused sieve tubes with wound gum. B. Mahaleb stock. The sieve tubes are collapsed to the cambium. (Both \times 110.)

Two trees which survived the first year they were inoculated and which were examined the following spring after a few large sieve tubes had been produced had no wound gum or callus in the winter or new spring phloem; but a strong reaction occurred in the previous summer's phloem.

There was a similar distribution of wound gum in buckskin-diseased Early Richmond cherries (*Prunus cerasus* Linn.) and in *P. molis* (Walp.) when on Mahaleb. The injury was most extensive below the graft unions.

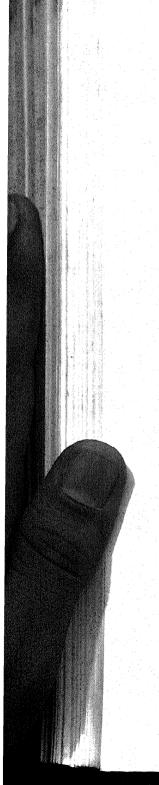
Histochemical studies have shown that the wound gum in the sieve tubes of diseased cherry has the same properties as that in diseased peach.

These observations perhaps indicate that the virus is elaborated in the Napoleon cherry tops and is then carried to the Mahaleb stocks by way of the phloem where severe necrosis results. The injury in the older phloem of the Napoleon tops is probably a secondary effect caused by necrosis of sieve tubes in the Mahaleb phloem. The injury was only apparent in the Mahaleb cherry phloem near the graft unions of trees in early stages of the disease, but it extended further down the trunk and roots in later stages.

Wound Gum in the Sieve Tubes of Diseased and Healthy Cherry Trees on Mazzard Roots

With the exception of one sample taken just beneath a canker in the cortex, buckskin-diseased and nonbuckskin cherry trees grown in orchards on Mazzard roots contained no wound gum. No wound gum was in branches from three trees suffering from zinc deficiency, in a branch of a tree suffering from bacterial gummosis, and in eight sections from branches and roots of five buckskin-infected trees. The absence of wound gum and phloem degeneration is in keeping with the relatively mild external symptoms in buckskin-diseased trees grown on Mazzard stocks.

Young Napoleon cherry trees on Mazzard roots which were grown in the greenhouse and were infected with the Green Valley and Palo Alto strains of virus had some wound gum in the phloem. In the Mazzard rootstock of one normal-looking tree which had been inoculated with Green Valley buckskin virus three years earlier, there were several bands of wound gum in the nonfunctioning phloem of previous years in the trunk, tap root, and branch roots. The nearest sieve tubes which contained wound gum were 400 microns from the cambium immediately below the bud union and 300 microns from the cambium at a point five inches below the bud union. The current season's phloem was apparently not affected. The Napoleon top at three levels had only occasional sieve tubes with wound gum, and they were 600 microns from the cambium at a point just above the union. Another normal-looking tree infected by the same virus gave a mild reaction in the outer margin of the summer phloem in a section a few inches from where a branch was inarched to a diseased sour cherry tree (Prunus cerasus). Above the bud union a reaction of medium intensity was obtained 400 microns from the cambium. The color was less intense below the bud union but occurred only 230 microns from the cambium. Six inches below the union in the tap root



the coloration was spotted, and it was absent in a lateral root. A normal-looking cherry tree on Mazzard root stock inarched to a peach tree infected with the Palo Alto virus showed only spotted reactions in five sections taken at points throughout the tree. The affected sieve tubes were 300 to 500 microns from the cambium. Another Napoleon tree carrying peach scions affected by the Palo Alto strain of virus on several top branches showed mild injury in the branches below the scions, medium injury in the trunk just below the branches, mild reactions above and below the bud union, and none at all in the roots. The reactions were observed 200 to 270 microns from the cambium in the outer summer phloem. Two of three sour cherries on Mazzard roots gave light reactions in stems of young greenhouse trees but little or no gum was present above and below the bud unions.

The results indicate that the diseased young sweet cherry trees on Mazzard stocks grown in the greenhouse were more susceptible to sieve-tube injury than large orchard trees. Possibly because of genetic variability, some Mazzard seedling root stocks showed more phloem injury than others. Also, several peach scions infected with Palo Alto virus caused more injury in the cherry being inoculated than was obtained with single cherry scions infected with Green Valley virus, or than was obtained by inarching the ends of branches of diseased trees to the trees being inoculated. The amount of injury observed in trees on Mazzard stock did not in any case approach that in sweet cherries on Mahaleb stock.

Two noninoculated Mazzard seedlings about three feet tall which were growing in five-gallon cans contained no wound gum in the sieve tubes although one of them was dying (apparently from bacterial gummosis). A healthy Napoleon tree on Mazzard roots showed no injury above and below the union, but occasional sieve tubes in the tap root contained wound gum.

Anatomy of Swollen Veins of Diseased Cherry

Leaves with swollen veins were collected from a buckskin-diseased Napoleon cherry tree on Mazzard roots in Green Valley, California, in August, 1941. Paraffin sections of a lateral vein showed that the swelling was caused by excessive amounts of new secondary xylem and phloem. The sieve tubes had very thick nacré walls and the metaphloem sieve tubes were partially crushed. About half of the cells between the phloem rays were sieve tubes and about half were parenchyma cells. Sieve plates of the sieve tubes in the older half of the phloem were callused.

Leaves from the healthy trees did not have the wide band of secondary tissue in the lateral veins, and the nacré walls of the metaphloem sieve tubes were very thick and almost filled the lumina of the cells. No definitive callus was present. Unlike the lateral veins of the peach, those of the cherry contained protophloem fibers.

DISCUSSION

The effect of the buckskin virus on the sieve tubes of the secondary phloem of peach trees and of sweet cherry trees when on Mahaleb rootstock

resembles that of the potato-leaf-roll virus on the primary sieve tubes of potato plants. The histology of plants infected by leaf-roll virus has been studied extensively. Esmarch (6) and Esau (5) reviewed the literature on the subject. As Esau has pointed out some of the workers have studied plants with a mixture of virus diseases and therefore their results are difficult of interpretation. Judging by the studies (1, 2, 13, 19) in which the authors have taken precautions to use plants with only the leaf-roll virus, the following changes occur in the sieve tubes and companion cells. The walls thicken and a separation between the primary walls occurs. Yellowing of walls and contents takes place and a red color is obtained when the sections are treated with phloroglucinol and hydrochloric acid. The sieve tubes are crushed by the turgor of adjacent parenchyma cells. Necrosis is confined to sieve tubes and companion cells and occurs in the older parts of the phloem.

Necrosis of sieve tubes of the secondary phloem in peach and in sweet cherry on Mahaleb stock resulting from the buckskin disease resembles the necrosis of the sieve tubes in the primary phloem in potato plants with leaf roll, except that no swelling of walls or separation of primary walls has been observed. In fact, the walls become thinner (Fig. 2).

Many virus diseases affecting the phloem affect parenchyma cells near the sieve tubes. Esau (3, 4) reports that in leaves of sugar beets affected by the curly-top disease parenchyma cells adjacent to the sieve tubes first become hypertrophied and parenchyma cells further removed are subject to hyperplasia. The hyperplastic cells develop into abnormal sieve tubes. These abnormal tissues may then become necrotic, and adjacent parenchyma cells undergo proliferation and division. Magee (12) in his studies on the bunchy top of banana also found that parenchyma cells adjacent to sieve tubes underwent hypertrophy and hyperplasia. Parenchyma cells are usually not affected in buckskin-diseased plants; although, in severe cases necrosis of the entire phloem and cortex may occur and a hyperplastic condition may arise as a result of abnormal activity of the cambium in leaves. Fiber initials may also become necrotic in young tissues.

A recently described virus disease, phloem necrosis, of tea may resemble buckskin in peach. Some cells of the phloem are said to be collapsed and yellow, whereas adjacent cells appear to be normal in the photomicrograph shown (7). Necrosis also occurs in the midribs of diseased tea leaves and from the photomicrograph it appears as though new abnormal secondary phloem is produced in a manner similar to that found in buckskin-diseased peach. In a later paper it was reported (8) that severe vein-swelling occurs. Necrosis is said to be visible macroscopically in the phloem area in more advanced stages when tangential slices are removed from large roots.

When sweet cherry trees on Mahaleb roots are infected, extensive sievetube necrosis results in the Mahaleb stocks but only a small amount of necrosis occurs in the sweet cherry tops. The injury in the Mahaleb is immediately below the bud union when symptoms first appear, but in advanced

stages in orchard trees it has been found a foot or more below the union. The tree eventually dies as a result of the disease. Although Mahaleb rootstocks have been considered virtually immune to buckskin virus and have been extensively used for this reason in orchards affected by the Green Valley strain of virus, it seems probable now that Mahaleb trees are really hypersensitive, suffering such extensive injury to the phloem that movement of the virus is inhibited. The distal portions of the Mahaleb roots of affected small trees with Napoleon tops are often killed before the top is entirely dead. However, this may be an indirect effect of the drastic reduction in functioning phloem between top and roots, chiefly in the upper portion of the Mahaleb stock.

Sweet cherries are not completely compatible with Mahaleb root stock (10) as is evidenced by their small size and shorter life as compared to trees on Mazzard stock. Sections above and below the bud union indicate that the area of phloem is larger in the sweet cherry than in Mahaleb cherry. Sieve tubes of the Mahaleb stocks form definitive callus earlier in the autumn than do the sieve tubes of the sweet cherry tops. These differences in available phloem for translocation may be the cause of necrosis observed in the sieve tubes of healthy trees and the seeming incompatibility in certain situations. It should be emphasized, however, that when trees are infected with buckskin virus the injury becomes much more pronounced and causes severe necrosis during the summer as well as in the autumn; and ultimately all of the Mahaleb sieve tubes are killed. In healthy trees most of the necrosis occurs in the Napoleon tops.

Callusing of sieve plates followed by subsequent collapse of sieve tubes can be caused by several agencies and treatments. They are ringing, grafting on incompatible rootstocks, environmental conditions leading to dormancy, introduction of dilute solutions of eosin (18), and presence of virus (buckskin virus in peach and cherry and potato-leaf-roll virus in potato). It seems probable that interference with translocation or the presence of a toxic substance may be the cause of sieve-tube necrosis, especially in case of virus infections. Presence of virus in one part of the phloem could cause necrosis, upset normal translocation, and lead indirectly to sieve-tube necrosis in other parts of the plant. Possibly this happens when diseased Napoleon cherries are on Mahaleb roots. Normally the sweet cherry sieve tubes are little affected by buckskin virus when on Mazzard roots, but when the sieve tubes of Mahaleb stock become necrotic the subsequent necrosis of sweet cherry sieve tubes may, at least in part, be a secondary effect due to the death of the Mahaleb sieve tubes. Products of metabolism rather than the virus itself may be the immediate cause of some of the symptoms seen in infected plants.

A positive test with phloroglucinol and hydrochloric acid for wound gum in sieve tubes may be of value as a diagnostic symptom for buckskin disease in peach. It would be of value, however, only if stems of large diameter were taken at some distance from injuries and from trees grown on a compatible rootstock. Branches showing leaf symptoms are most apt to give a positive test. A positive test should be considered only as another symptom of buckskin disease and not specific proof of it without accessory evidence.

SUMMARY

Necrosis of sieve tubes occurs in buckskin-diseased peach trees. Necrotic sieve tubes contain a substance which is here called wound gum. This substance gave a positive test with phloroglucinol and hydrochloric acid and a negative, or a weakly positive, reaction with the Maule reagents for lignin. It was insoluble in a mixture of potassium chlorate and hydrochloric acid, and in hot 10 per cent nitric acid. After the wound gum was removed by alternate treatments with chlorine water and sodium sulphite, the sieve tubes gave a positive test for cellulose although they did not do so before the wound gum was removed. Usually necrosis was limited to the sieve tubes, but sometimes it also involved other cells.

In young peach shoots with early stages of necrotic spotting in the leaves, necrosis of sieve tubes was confined to leaves with symptoms and to the leaf traces below the affected leaves. As the virus spread into the stem tips, sieve tubes of the youngest leaf primordia which had developed these elements became necrotic. The oldest sieve tubes became necrotic first. If, after the onset of disease, growth was resumed, new sieve tubes usually failed to undergo turgor expansion, lacked nacré walls, and early became necrotic.

When certain strains of the buckskin virus or very susceptible peach varieties were used, cankers developed on the stems. The cankered areas contained gum pockets in the cortex and phloem. Such areas were isolated by periderms. Gum pockets in some instances appeared in the unlignified new xylem.

Swollen veins in infected peach were characterized by abnormal production of phloem. Sieve tubes of this abnormal phloem soon became necrotic, as did also those of the original phloem.

Ringing of the stems caused swollen veins to develop in peach. Sieve tubes in the stems immediately above the rings collapsed and small amounts of wound gum sometimes developed in them. New phloem was formed in which the sieve tubes failed to undergo turgor expansion. Gum pockets formed in newly initiated unlignified xylem.

Peach scions on Myrobalan root stock often grow normally at first, but later exhibit yellow, rolled leaves with swollen veins and some premature defoliation. Such stems contained necrotic sieve tubes with wound gum. New phloem was initiated in which sieve tubes did not undergo turgor expansion.

In uninfected young sweet cherry trees on Mahaleb stocks grown in pots, occasional sieve tubes on the outer margin of the current year's summer phloem and the non-functioning phloem of previous seasons in some cases contained wound gum. In the summer phloem of healthy orchard trees wound-gum formation occurred frequently in the autumn just before trees

became dormant. In autumn the Mahaleb sieve tubes were observed to form callus before the sweet cherry sieve tubes. Wound gum was more abundant in the sweet cherry tops than in the Mahaleb stock. The reverse was true in buckskin-infected trees.

In infected cherry trees on Mahaleb stock, wound gum formation was very extensive even in midsummer in the summer phloem. Ultimately the youngest sieve tubes were affected in the Mahaleb stock just below the bud unions.

No injury to phloem has been observed in either diseased or healthy orchard trees on Mazzard roots. Some wound gum was present in the outer phloem in infected young greenhouse trees on this stock.

DIVISION OF PLANT PATHOLOGY. UNIVERSITY OF CALIFORNIA. BERKELEY, CALIFORNIA.

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A ROOT ROT OF GUAYULE CAUSED BY PYTHIUM ULTIMUM

W. A. CAMPBELL AND BAILEY SLEETH (Accepted for publication March 10, 1945)

INTRODUCTION

A root rot of guayule (Parthenium argentatum Gray) nursery seedlings from which a Pythium was consistently isolated was reported as prevalent in the nurseries at Salinas, California, in 1942. The following year there was a re-occurrence of the disease and it was also reported from the nurseries near Oceanside,² California. Its prevalence in the nurseries at Salinas in 1944 was limited because of the small acreage planted. In both 1943 and 1944 the disease was found in direct field seedlings in the Salinas Valley.

The causal fungus, identified as Pythium ultimum Trow, attacks the roots of young guayule seedlings over a considerable time and causes characteristic symptoms. When the roots of plants in the cotyledon stage are affected, the disease is termed "seedling root rot." If the tap roots or root crowns of older seedlings are attacked and the woody cylinder at the lesion is pinkish or reddish, the disease is called "pink rot." The loss from seedling root rot was usually greater than the loss from the pink rot stage of the disease which occurred on older plants.

SEEDLING ROOT ROT

Seedling root rot of guayule may develop shortly after emergence and continue to affect the plants in the cotyledon stage or during a period of approximately four weeks. The first observed symptom is a slight stunting associated with a purplish or reddish color of the cotyledons. The roots of affected plants are rotted at varying depths in the soil and the degree of stunting depends upon the extent of injury to the root systems (Fig. 1, A). Lesions that develop on the tap root near the soil surface usually result in severe stunting or the death of the seedlings. Infections which develop deeper in the soil and which are limited to a few lateral roots may not greatly affect the growth rate or appearance of the plants. The fungus may invade and destroy most of the root system or be confined to a small area at the point of infection. Because new lateral roots develop above the lesion on the tap root, a plant affected by seedling root rot usually has a forked or multiple tap root.

Seedling root rot of guayule may be regarded as a part of the damping-off disease complex with symptoms sufficiently distinct to separate it from post-emergence damping-off. Infected seedlings, even though much of their root systems may be destroyed, usually survive and under good growing conditions tend to overcome the initial loss of roots. The recovery of diseased seedlings from fungus attack is associated with a reduction in

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soil moisture in the upper 2 to 3 inches of soil and an increase in the age of the seedlings. Ordinarily, satisfactory emergence is obtained in 10 to 14

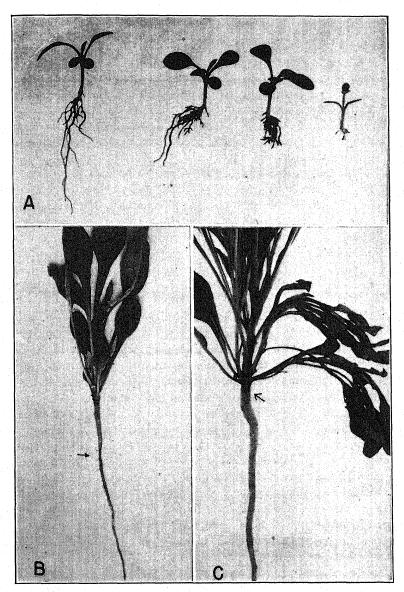


Fig. 1. Pythium root rot of guayule. A. Seedling root rot on very young plants. New root development has been initiated above the diseased parts in all four seedlings. (Approximately natural size.) B and C. The pink rot stage of Pythium root rot. The arrows point to lesions on the tap root; (B) at some distance below the soil surface; and (C) at the root crown. (Approximately one-half natural size.)

days after sowing, after which the frequency of irrigation is reduced and the soil moisture conditions become less favorable for fungus infection near the soil surface.

PINK ROT

The pink rot stage of Pythium root rot of guayule seedlings ordinarily develops on plants 6 to 16 weeks old. The first evident symptom of the disease is wilting during the heat of the day. The severity of wilting and eventual death or recovery of affected plants depends upon the location and extent of the lesions on the tap roots. Plants with circumferential lesions on the upper tap root or at the root crown usually do not recover; those with lesions on the lower tap root deep in the soil wilt slightly during the warm part of the day but recover in a few days. The small feeder roots may be attacked as well as the tap root. Injury to the small roots ordinarily does not cause the plants to wilt because of the number of uninfected roots available to supply moisture to the top.

Pink rot lesions on the tap root are rarely more than $\frac{1}{8}$ to $\frac{1}{2}$ inch long (Fig. 1, B), and occur most frequently 3 to 6 inches below the soil surface. In the early stages the infected tissues are watersoaked, translucent, and somewhat pinkish. Later the diseased bark becomes soft and depressed and it usually sloughs off when the plant is pulled from the ground. The woody cylinder underneath the lesion and for a short distance above and below is pinkish or reddish. This characteristic color serves as a diagnostic aid in separating root rot caused by *Pythium ultimum* from that caused by other fungi.

Infections which develop at or slightly above the root crown are commonly associated with the piling of earth against the lower leaves and stems of the plants by cultivators or by machines used to apply fertilizer. After such operations, provided the beds are irrigated, there is usually a sharp increase in the number of pink-rotted plants which reaches a peak within 3 or 4 days. Crown infections were induced experimentally by piling earth against the plants. The number of plants infected was proportional to the depth to which the soil was piled against the plants and to the amount of irrigation water applied. There is a tendency for lesions at the root crown to increase during prolonged periods of foggy weather. Crown lesions in plants from 12 to 16 weeks old often cause the progressive death of the top branches; and such plants in the early stages of the disease may be recognized by the presence of conspicuous "flags" (Fig. 1, C).

Pink rot was observed most frequently on the heavier types of soil. Instances of severe and extensive losses from pink rot followed 4 to 6 hours of continuous irrigation with the overhead sprinkling system on heavy soil. Also, losses were severe on low areas where water accumulated from irrigations of shorter duration. Losses were relatively unimportant on the lighter and well-drained soils.

THE CAUSAL FUNGUS

The association of *Pythium sp.* with seedling root rot and pink rot of guayule was established in the course of routine isolations from diseased plants. The pathogenicity of the fungus was demonstrated by pure culture

inoculation tests made in the greenhouse, and its identity as *Pythium ulti-mum* Trow confirmed by comparison³ and examination of 51 isolates.

The macroscopic appearance and growth rates of the 51 isolates were similar in culture. However, only 27 isolates produced oogonia and antheridia on corn-meal agar. The remaining 24 isolates produced only sporangia or sporangia-like structures. All attempts to obtain oogonia and antheridia by growing these isolates on the culture media usually used for this purpose were unsuccessful.

The 27 isolates that produced oospores in culture agreed in all essential respects with an isolate from guayule identified by John T. Middleton as Pythium ultimum Trow. Although many of the isolates failed to produce oospores in culture, they were considered as referable to P. ultimum because of their similar morphological appearance and comparable rate of growth. This conclusion was strengthened by the common source of the isolates in that they were obtained from the same diseased material. This viewpoint is in agreement with Tompkins, Ark, Tucker, and Middleton⁴ who reported the isolation of a strain of P. ultimum from Zucchini pumpkin fruits which did not produce oospores. Furthermore, there was a progressive gradation in the isolates from those that produced an abundance of oospores and few sporangia to those that produced only sporangia or sporangia-like structures. This behavior in respect to spore formation is suggestive of the dual phenomenon of Hansen⁵ which is common in imperfect fungi but which has not been demonstrated in the Phycomycetes.

SUMMARY

Pythium ultimum Trow was determined as the causal agent of a root rot of guayule (Parthenium argentatum Gray) nursery seedlings in California. Two stages of the disease are described; seedling root rot which affected plants in the cotyledon stage and "pink rot" which affected plants from 6 to 16 weeks old. The over-all losses in the nurseries were not serious, however, the disease occasionally caused considerable mortality in localized areas especially on heavy poorly drained soil.

Twenty-seven of the 51 isolates of the fungus produced oospores in culture and were considered typical *Pythium ultimum*. Twenty-four of the isolates produced only sporangia or sporangia-like structures in culture but were referred to *P. ultimum* on basis of association with oospore-producing isolates, morphological appearance, and growth rates.

SPECIAL GUAYULE RESEARCH PROJECT.

SALINAS, CALIFORNIA.

3 Middleton, John T. The taxonomy, host range, and geographic distribution of the

genus Pythium. Memoirs, Torrey Bot. Club 20: 1-171. 1943.

4 Tompkins, C. M., P. A. Ark, C. M. Tucker, and J. T. Middleton. Soft rot of pumpkin and watermelon fruits caused by *Pythium ultimum*. Jour. Agr. Res. [U.S.] 58: 461-475. 1939.

⁵ Hansen, H. N. The dual phenomenon in imperfect fungi. Mycologia 30: 442-455. 1938.

A SIMPLE METHOD OF INOCULATING BARLEY WITH LOOSE SMUT¹

J. M. POEHLMAN

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Winter barley varieties commonly grown in Missouri are highly susceptible to loose smut, *Ustilago nuda* (Jens.) Rostr. The breeding of winter types resistant to this disease has been handicapped by the lack of information regarding the relative resistance of common varieties and the scarcity of parent material with high resistance. Much of this lack of information results from difficulties encountered in the artificial inoculation of breeding material. In the winter of 1942–43, 708 varieties of barley, representing the winter types in the U. S. Department of Agriculture barley C.I. collection, were grown at Columbia, Missouri. Many of these varieties were introductions from foreign lands and little or nothing was known regarding their reaction to loose smut. The possibility that some winter types with good resistance to loose smut could be found, led the author to carefully survey the material at hand.

The partial vacuum technique for inoculating barley with loose smut as described by Moore (3) and used by other workers, (1, 2, and 4), had been used by the author in the previous season with fair success. Three heads from each of 40 varieties and selections of winter barley had been inoculated. The percentage of smutted heads from the inoculated seed (average of 40 varieties) was 22.6 per cent as compared to 0.6 per cent in uninoculated But several objections to this method were evident. ment was clumsy and the procedure time-consuming when employed in the The early flowering of barley, sometimes even before emergence from the boot, made it necessary to inoculate while the culms were very tender. Injury to the culms or even breakage frequently occurred. Failure of the glumes to open properly when the vacuum was applied sometimes resulted in poor inoculation. Because inexperienced help was used for this work, a simple, positive and efficient method of inoculation, that could be quickly learned, was necessary if many varieties were to be tested.

Some preliminary inoculations made in preceding years in the field and in the greenhouse suggested a simple procedure that could be successfully used. The method and the results from its use are described.

DESCRIPTION OF METHOD

The equipment used consisted of a hypodermic needle (1 inch, 25 gauge) inserted into a rubber bulb (10 cc. capacity). The bulb was filled with a suspension of chlamydospores of *Ustilago nuda*. A few drops of this suspension was injected into each floret by piercing the lemma with the needle and squeezing the bulb slightly. Inoculations were made one to two days after

the spike broke the boot. At this stage the majority of the florets had reached or just passed pollination. Immature florets at the base of the spike were removed. If the variety was awned, the awns were usually clipped to facilitate inoculation but this was not necessary. After inoculation each spike was tagged for identification. Spikes inoculated in this manner developed normally with no apparent injury and with little if any reduction in seed setting except from the removal of the immature spikelets.

The suspension of chlamydospores was prepared in the field from fresh Adjacent field plots in 1943 contained ten varieties of winter barley, all of which were infected with loose smut (Ustilago nuda) in varying amounts. These barleys had been grown from commercial lots of seed obtained from several locations in Missouri, Oklahoma, Indiana, or Ohio. This offered an opportunity to use inoculum of widely scattered origin. Each day smutted heads were selected from all of these varieties, chopped into small pieces, placed in a small square of cheese cloth, and immersed in tap water. The smut spores were strained through the cheese cloth similar to the manner in which a tea bag is used. By this method a concentrated spore suspension could be prepared without inclusion of extraneous plant materials that might clog the hypodermic needle. Sufficient dextrose was added to the suspension to make a one per cent solution and the contents were shaken vigorously.

The rubber bulbs held sufficient inoculum to inoculate 15–20 separate spikes. As the bulbs fill slowly, an extra bulb was carried in a beaker of suspension by each worker to avoid loss of time. Varieties and selections to be inoculated were listed on index cards to which were attached envelopes containing a labeled tag for each head to be inoculated. Tags of a specific color were used for labeling of heads inoculated with smut so that they could readily be identified. After some skill had been attained, 30 to 40 heads could be inoculated per hour by a worker.

Three heads were inoculated from each variety or selection. These three heads, along with two uninoculated heads, were harvested, each head threshed separately and the seed planted in a one-foot row in the field in the fall of 1943. Removal of the immature spikelets reduced the number of seeds from each head but by planting in a one-foot row a dense stand could be obtained. Previous experience had demonstrated that thinly spaced plants on the soil type present at Columbia, Missouri, frequently heave badly and would thus be lost. This rate of planting precluded taking notes on individual plants so counts of normal and smutted heads were made in each row the following spring. Percentages of smutted heads in each variety were calculated as the average from the three rows.

MATERIAL INOCULATED AND RESULTS

Three groups of material were inoculated as follows:

(1) 180 selections from the C.I. winter barley group (representing all selections from the 708 varieties that survived the winter at Columbia, Missouri, with a stand of 60 per cent or better).

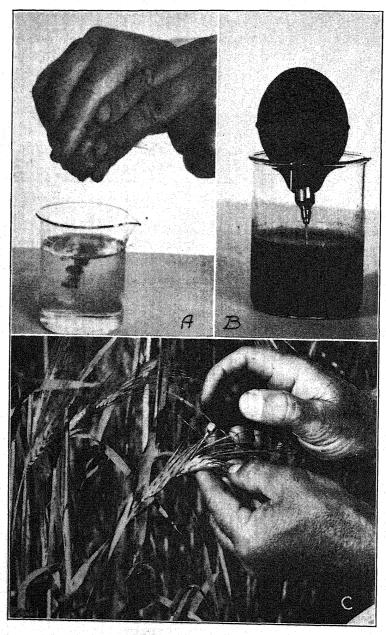


Fig. 1. Steps in the inoculation of barley with loose smut. A. Preparation of inoculum by straining smut chlamydospores through cloth and making a spore suspension in tap water plus dextrose. B. The rubber bulb fitted with hypodermic needle and ready for filling with inoculum. C. Inoculation of the barley florets.

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- (2) 42 standard varieties from nursery and uniform winterhardiness tests.
- (3) 20 selections from the variety Missouri Early Beardless. Average percentages of infection, as determined by counts of smutted heads, in inoculated and uninoculated check rows of each group are listed here: (1) C.I. group, inoculated, 23.2 per cent, uninoculated checks, 1.5 per cent; (2) standard variety group, inoculated, 22.1 per cent, uninoculated checks, 3.6 per cent; and (3) Early Beardless selections, inoculated, 10.2 per cent, uninoculated checks, 2.4 per cent. The distribution of the barley selections from these groups in infection percentage classes is summarized in table 1.

The percentages of smutted heads in the inoculated material as compared to percentages in the uninoculated checks demonstrate the effectiveness of the method. In the C.I. barley group of 180 selections (Table 1), 80 selec-

TABLE 1.—Distribution of the barley selections in infection percentage classes

	Infection percentage classes								
Material —	0		0.1-20	20.1-40	40.1-60	60.1-100			
C.I. barley group:						and the second s			
No. of selections	42		58	38	27	15			
Per cent of total	23.3		32.2	21.1	15.0	8.3			
Standard variety group:									
No. of selections	- 8		14	12	7	1			
Per cent of total	19.0		33.3	28.6	16.7	2.4			
Early Beardless selections:									
No. of selections	8		7	4	1	*******			
Per cent of total	40.0		35.0	20.0	5.0	10,100			

tions (44.4 per cent) contained over 20 per cent smutted heads, 42 (23.3 per cent) contained over 40 per cent smutted heads, and 15 (8.3 per cent) contained over 60 per cent smutted heads. A similar distribution is noted in the standard variety group. Resistance had previously been recorded in the Early Beardless selections, so lower infection percentages were expected in this group.

The number of selections in the C.I. group without smut (23.3 per cent) appears proportionally large. Of the 42 selections without smut, 11 had plants surviving from only two of the inoculated rows, while 13 had plants surviving from only a single inoculated row. All of these 24 selections were low in vigor and in each winter killing had been severe. That plants infected with loose smut are more susceptible to winter injury is suggested by winter killing notes on the inoculated and uninoculated rows. Survival in the inoculated rows was eight to ten per cent less than in the uninoculated checks. Similar results have been reported by other investigators (4, 5). In the 24 weak selections, infected plants may have been lost from winter injury. These may therefore represent escapes rather than resistant types.

Of the eight standard varieties reported without infection (Table 1), all are known to possess some resistance. In four of these, loose smut has not been observed previously in the nursery at Columbia, either from natural

Two of the selections have previously contained or artificial inoculation. small amounts of smut from natural infection. The other two varieties had not been grown at Columbia before. In three of the eight Early Beardless selections without infection, loose smut has never been observed either from natural or artificial inoculations, while in the other five slight infections from artificial inoculations or traces from natural infections have previously been observed.

All selections containing five per cent or less infection were reinoculated. Detailed reactions will be reported when these selections have been more fully checked.

DISCUSSION AND SUMMARY

A simple method of inoculating barley with loose smut (*Ustilago nuda*) is described. Infections obtained by this method in a wide range of barley varieties demonstrate that it may be successfully used to sort out susceptible and resistant varieties. Percentage infections in individual varieties inoculated by this method are in the range previously obtained by the partial vacuum technique. The simplicity of the method described makes it adapted for field inoculation of large numbers of varieties or selections in a breeding It should prove useful for the plant breeder in finding barley varieties resistant to loose smut or in selecting resistant lines from segregating material.

DEPARTMENT OF FIELD CROPS. UNIVERSITY OF MISSOURI. COLUMBIA, MISSOURI.

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SMUT CONTROL IN SORGHUM AND EFFECT OF DUST FUNGICIDES AND STORAGE ON EMERGENCE¹

R. W. LEUKEL AND J. E. LIVINGSTON2

(Accepted for publication March 26, 1945)

Excellent control of covered kernel smut (Sphacelotheca sorghi (Lk.) Clint.) in Sharon kafir was obtained in field experiments at 7 stations in 1942^3 by the use of several dust fungicides at the generally recommended dosages, and at $\frac{1}{2}$ and $\frac{1}{4}$ of these dosages. The relatively low average percentage of smutted heads (25.3 per cent) from untreated seed rendered those results somewhat inconclusive and made it desirable to obtain more data on the control of kernel smut by some of these fungicides and their effect on emergence, especially when treated seed is stored.

Similar tests, therefore, were carried out at 5 stations in 1943 and at 2 stations in 1944. Several of the fungicides used in 1942 were replaced in 1943 by others, some of which proved unsatisfactory and were replaced by still others in the 1944 tests.

MATERIALS AND METHODS

The following fungicides were used:

- 1. N.I. (New Improved) Ceresan (5 per cent ethyl mercury phosphate).
- 2. Copper carbonate, containing 50 per cent metallic copper.
- 3. Spergon (98 per cent tetrachloro parabenzoquinone).
- 4. Arasan (50 per cent tetramethylthiuramdisulfide).
- 5. DuBay 1452-C (6.5 per cent ethyl mercury p-toluene sulfonanilide).
- 6. Leytosan (7.2 per cent phenyl mercury urea).
- 7. Merc-o-dust, a compound of uncertain composition purported to contain 1.5 per cent mercury and 2 per cent formaldehyde.
 - 8. M.T.D.S. (morpholine thiuramdisulfide).
 - 9. Fermate (70 per cent ferric dimethyl dithiocarbamate).
 - 10. U.S.R. 604 (dichloro naphthoquinone).
 - 11. Zimate (zinc dimethyl dithiocarbamate).
 - 12. Basic copper sulfate, containing 50 per cent metallic copper.
 - 13. Sulfur (300-mesh dusting sulfur).

Numbers 1, 2, 3, 4, and 13 were used both years. Numbers 5, 6, 7, and 8 were used in 1943, and 9, 10, 11, and 12 in 1944 only. Seed of Leoti sorgo and Sharon kafir was used both years, the first because it is susceptible to seed injury by certain fungicides, and the second because it is highly sus-

¹ Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nebraska Agricultural Experiment Station.

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² Pathologist, Division of Cereal Crops and Diseases and Assistant Pathologist, Nebraska Agricultural Experiment Station, respectively.

³ Leukel, R. W. New fungicides and reduced fungicide dosages for the control of kernel smut of sorghum. Phytopath. 32: 1091-1093. 1942.

ceptible to covered kernel smut and has an upright, low habit of growth which greatly facilitates taking data on smut control. The seed after being thoroughly cleaned was dusted with viable spores of covered kernel smut at a 1 to 100 spore dosage (by weight) after which separate portions were treated with the different fungicides to be tested, and planted in field plots in triplicated 44-foot rows at the rate of 200 seeds per row. Stand counts were made after the plants had had ample time to emerge. Data on smut control were taken by counting the total number of heads and the smutted heads in each row.

TABLE 1.-Effect of seed treatment with certain dust fungicides on emergence in two varieties of sorghum planted at the rate of 200 seeds per 44-foot row at several stations in 1943

Seed treatment										
Material	Rate	220002 501 80								
		Avere	Difference	Odds	Aver.d	Difference	Odds			
	Oz.	Pct.			Pct.					
New Improved Ceresan	$\frac{1}{2}$	68.8	20.8 ± 4.5	1:9999	39.4	-9.9 ± 1.7	*			
do	Ī	65.9	17.9 ± 2.9	*	49.6	0.3 ± 3.9	1: 1			
Copper carbonate	\cdot $\hat{2}$	69.2	21.2 + 2.3	*	55.1	5.8 ± 2.6	1: 35			
do	1	64.9	16.9 ± 2.7	*	48.8	-0.5 ± 1.3	1: 2			
Spergon	2	62.4	14.4 ± 3.4	1:2998	47.3	-2.1 ± 3.8	$\frac{1}{1}$: 2			
do	1	59.5	11.5 ± 3.2	1: 627	48.5	-0.8 ± 2.4	1: 2			
Arasan	2 .	65.3	17.3 ± 2.3	*	57.7	8.4 ± 2.8	1: 133			
do	1	62.4	14.4 ± 3.1	1:9999	55.9	6.6 + 2.4	1: 62			
DuBay 1452-C	1	67.7	19.7 ± 2.6	*	34.8	-14.5 + 1.9	*			
do	Ĩ	66.3	18.3 ± 2.5	*	57.8	8.4 ± 2.7	1: 169			
Leytosan	į	59.2	11.2 ± 1.8	*	52.6	3.3 ± 3.7	1: 103			
do	i	59.3	11.3 + 1.9	*	49.3	0.0 ± 3.7	1: 1			
Morpholine thiuramdi-	**		22.0		10.0	0.0 ± 3.3	11. 1			
sulfide	2	57.5	9.5 ± 2.5	1:1110	48.8	-0.5 ± 2.9	1: 1			
do	1	54.8	6.8 ± 2.2	1: 244	50.8	1.5 ± 2.8	1: 1: 2			
Merc-o-dust	1	49.0	1.0 ± 2.1	1: 2	43.7	-5.7 ± 1.6	1: 293			
do	į	(M. O.	-0.1 ± 1.8	ī: ī	47.7	-3.7 ± 1.0 -1.7 ± 0.5	1: 293			
Bulfur	$\tilde{2}$	46.0	-2.0 ± 2.0	1: 5	35.3	-1.7 ± 0.5 -14.0 ± 2.6				
do	1	44.0	-4.0 ± 2.2	$\overline{1}$: 16	41.2	-8.2 ± 3.5	1:4999			
Check		48.0	1.0 _ 2.2		49.3	- 0.4 ± 3.5	1: 40			

* Odds greater than 1: 9999.

Three replications planted at each of 6 locations.

e Each figure is based on 3600 seeds planted.

EXPERIMENTAL RESULTS

In 1943, separate lots of smutted seed of Sharon kafir and Leoti sorgo were treated with 9 fungicides at two rates of application. Sharon kafir was planted at five stations4 and Leoti at three stations. Two plantings were made at Lincoln, Nebraska, one on May 22 and another on June 17. The data on emergence and on the control of covered kernel smut are summarized in tables 1 and 2, respectively.

The seed was planted and the data on emergence and smut control taken by the following: E. G. Heyne, Manhattan, Kansas; A. F. Swanson, Hays, Kansas; A. E. Lowe, Garden City, Kansas; and O. J. Webster, Lincoln, Nebraska. Their kind cooperation is gratefully appreciated.

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The data on emergence were sufficiently similar at the different stations to be analyzed as a single experiment, using Student's method⁵ to compare the results from each lot of treated seed with results from untreated seed. Emergence in Sharon kafir was significantly improved by 7 of the fungicides. Emergence in Leoti was significantly impaired by N.I. Ceresan, DuBay 1452–C, Merc-o-dust, and sulfur applied at the generally recommended rates of treatment, and significantly improved by Arasan applied at 2 ounces, and DuBay 1452–C at $\frac{1}{4}$ ounce per bushel.

TABLE 2.—Control of covered kernel smut in Sharon kafir and Leoti sorgo, grown from seed that had been inoculated at a 1 to 100 spore dosage, treated, and planted at several stations in 1943

Seed treatment		Percentage smutted heads in											
Fungicide	Rate per		Sharon kafir at						Leoti sorgo at				All
	bu.	G.C.	H.	В.	L-1	L-2	М.	sta- tions	В.	L-1	L-2	м.	sta- tions
-4	Oz.												
None	*****	33.8	3.3	24.3	37.3	27.9	14.1	23.5	10.0	19.5	6.1	13.0	12.2
N.I. Ceresan	$\frac{1}{2}$	0.2	0.0	0.0	0.0	0.7	0.0	0.2	0.0	0.0	0.0	0.0	0.0
do	4	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Copper carbonate	2	0.0	2.1	0.0	0.0	0.0	0.0	0.4	0.4	1.2	0.0	1.6	0.8
do	1	0.0	1.1	0.0	0.0	0.0	0.0	0.2	0.4	1.3	2.4	0.6	1.2
Spergon	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
do	$\frac{1}{2}$	0.0	0.0	0.3	0.0	0.0	0.0	0.1	0.8	0.0	0.3	0.3	0.4
Arasan	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
do	$\frac{1}{2}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.2
None		36.4	3.7	22,9	32.9	24.5	6.0	21.1	14.1	25.0	6.6	10.5	14.1
DuBay 1452-C	1/2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0	0.0	0.7
_ do	1	0.4	1.1	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Leytosan	1/2	0.0	0.0	0.0	0.7	1.4	0.0	0.4	0.0	0.3	0.0	0.0	0.1
do	1	0.8	0.0	0.0	0.0	0.7	0.0	0.3	1.7	5.2	1.2	2.1	2.6
M.T.D.S	1	0.0	0.0	0.0	0.4	0.0	0.0	0.1	0.9	2.4	0.4	2.5	1.6
do	$\frac{1}{2}$	0.2	0.0	1.0	1.7	0.7	0.4	0.6	1.1	7.1	1.9	1.6	3.0
Merc-o-dust	$\frac{1}{2}$	35.3	3.6	30.9	41.7	24.2	7.0	23.8	16.3	14.2	4.0	9.0	11.0
do	$\frac{1}{4}$	40.1	5.6	26.8	52.0	20.3	10.0	25.8	8.6	20.2	6.2	8.2	10.8
Sulfur	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.4	0.2
do	1	0.7	0.0	0.0	0.5	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.1

^a Stations were: G.C.—Garden City, Kans.; H.—Hays, Kans.; B.—Beltsville, Md.; L-1 and L-2—Lincoln, Nebr. (2 plantings); and M.—Manhattan, Kans.

The percentage of heads infected with smut was again somewhat low for obtaining conclusive data on the fungicidal effectiveness of the treatments. In Sharon kafir the smut was controlled fairly well by all fungicides except Merc-o-dust, which proved worthless as a fungicide in both varieties. In Leoti an average of more than 1 per cent of the heads was smutted when grown from seed treated with copper carbonate (1 ounce), Leytosan (\frac{1}{2} ounce), and M.T.D.S. (1 ounce and \frac{1}{2} ounce).

Four of the fungicides used in 1943, namely, DuBay 1452-C, Leytosan, M.T.D.S., and Merc-o-dust, were replaced by Fermate, U.S.R. 604, Zimate, and basic copper sulfate in the 1944 experiments. These, along with the

⁵ Student, Biometrika 6: 1-25, 1908 and 11: 414-417, 1917.

other five used in 1943, were applied at 2 rates to smutted seed of Sharon kafir and Leoti sorgo. Sharon kafir was planted at Beltsville, Maryland, and at Lincoln, Nebraska. Leoti sorgo was planted at Beltsville for studies on emergence. The data on emergence and smut control are presented in table 3. Arasan was somewhat superior to the other dusts with respect to its effect on emergence, although U.S.R. 604, on the whole, was almost equal to Arasan in this respect, and at Lincoln was superior to it. At Beltsville

TABLE 3.—Effect of dust fungicides on emergence in sorghum and on control of covered kernel smut in 1944

Constant			Average	standa p	er row in		
Seed treatment	•	Sha	ron kafir	at		Smutted	
Fungicide	Rate per bu.	Lin- coln	Belts- ville	Lin- coln and Belts- ville ^b	Leoti at Belts- ville	Both varie- ties	heads at Belts- ville
	Oz.	No.	No.	No.	No.	Pct.	Pct.
Check (untreated) N.I. Ceresan		75 88	106 137	92 1 13	69 75	$\frac{41.7}{50.0}$	$61.3 \\ 0.0$
do	Ī	82	133	100	117	55.3	1.7
Copper carbonate	$\frac{2}{1}$	101	125 140	113 113	102 103	54.7	0.2
do Spergon	2	86 82	125	104	98	54.8 50.8	$0.3 \\ 0.0$
do		103	116	109	85	50.7	0.0
Arasan		100	151	126	152	67.2	1.1
do		86	151	119	144	63.5	5.7
Sulfur		75	121	98	75 	45.2	2.7
do Fermate	-	65 81	96 131	79 106	53 111	$35.7 \\ 53.8$	$10.5 \\ 5.5$
do		96	119	107	97	52.0	13.9
U.S.R. 604		110	137	124	130	62.8	0.6
do	-	100	147	107	129	62.7	0.0
Zimate		90	132	111	121	57.2	3.2
do	. 1	97	133	115	101	55.2	1.3
Basic copper sulfate		92	121	107	93	51.0	0.6
do	1	97	132	114	95	54.0	4.2
Least significant dif-		20	18	14	16		

Each bold face figure is significantly greater or less than the corresponding check.
 Data from Lincoln and Beltsville combined—each figure represents the average emergence per row in 6 rows with 200 seeds each.

c Smut infection at Lincoln was negligible.

all the treatments except sulfur improved emergence significantly following one or both rates of application used.

Not enough smut developed in the checks at Lincoln to obtain data of value on smut control. At Beltsville, however, the check rows averaged 61.3 per cent smut and this was reduced to less than 1 per cent by 5 of the fungicides, namely, copper carbonate, U.S.R. 604, and Spergon at both rates of application and by N.I. Ceresan and basic copper sulfate at the recommended rates. Arasan, which reduced the number of smutted heads to 1.1 per cent and 5.7 per cent at the 2 and 1 ounce rates, respectively, proved

somewhat less effective in smut control than in previous years. Hansing and Melchers⁶ report excellent control of smut in kafir with Arasan in experiments in 1943 in which 56.8 per cent smut appeared in the checks. N.I. Ceresan, DuBay 1452–C, Spergon, and sulfur also proved effective in their tests.

In field experiments near Wilmington, Delaware, in 1944, seed of each of seven varieties or crosses of sorghum was smutted with the different strains of covered kernel smut to which each of these varieties is susceptible, and then the seed was treated with different fungicides one day before planting in 25-foot rows, replicated 5 times. The data on smut control are shown in table 4. Infection in the checks ranged from 21.9 per cent in Leoti to 88 per cent in Kafir × feterita K. B. 2423.

TABLE 4.—Control of 5 races of covered kernel snut in different varieties of sorghum

		Percentage smutted heads from seed untreated or treated with								
Variety	Race of smut	Un- treated	N.I. Cere- san ½ oz.	DuBay 1452-F ½ oz.	Spergon ½ oz.	Arasan 1 oz.	CuCO ₃			
White Yolo	S-2	33.6	0.0	0.0	0.0	0.0	0.0			
do	S-4	85,6	0.0	0.0	Trace	3.1	Trace			
Scarborough broomcorn	S-1	75.7	$^{2.2}$	0.0	48.2	39.3	31.7			
Kafir x feterita KB 2423	S-3	80.3	0.0	0.0	0.0	4.9	1.7			
do	S-5	88.0	0.0	0.0	0.0	4.4	0.0			
Colby mile	S-2	32.3	0.0	0.0	0.0	0.3	0.0			
Cody	Mixture	40.4	0.0	0.0	0.0	4.0	1.9			
Leoti sorgo	S-1	21.9	0.0	0.0	2.4	7.9	3.3			
Sharon kafir	S-1	30.6	0.0	0.0	Trace	2.5	0.8			

With one exception (Scarborough broomcorn) the two volatile mercury dusts, N.I. Ceresan and DuBay 1452–F, applied at ½ ounce per bushel eliminated all smut from all 7 varieties. Spergon applied at the same rate controlled smut effectively in all but two varieties, Scarborough broomcorn and Leoti sorgo, both of which have a large proportion of seeds with persistent glumes. Copper carbonate, applied at 3 ounces per bushel reduced infection to less than 1 per cent in 5 of the 9 tests, and Arasan, at 1 ounce per bushel, was similarly effective in only 2 tests. These results suggest the advisability of using volatile mercury dusts for treating very smutty seed or seed with persistent glumes. The failure of Arasan to effect better control of smut possibly may be attributed to the short period between treating and planting.

Studies on the effect of treating sorghum seed on its germination after different periods of storage were started in December, 1943. Separate portions of seed (500 g.) of each of five varieties were treated with N.I. Ceresan

⁶ Hansing, E. D., and L. E. Melchers. Standard and new fungicides for the control of covered kernel smut of sorghum and their effect on stand. Phytopath. 34: 1034-1036. 1944.

Table 5.—Effect of seed treatment and subsequent storage of treated seed on emergence at 20° C. in sorghum planted in unsterilized soil

		E	merge	nce af						
Variety and treatment ^a -	2	20	0° C. 1			10° C. for 180	30° C. for 180	Average emer- gence	Differ- ence	*Odds
			days			days	days			
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.		
Club kafir Check	31	41	42	35	28	41	33	36		
Arasan	76	85	83	83	82	82	85	82	46 ± 4.0	*
Copper		- 00			0-		00		20 2 2.0	
carbonate	52	58	66	70	64	63	57	61	25 ± 2.5	*
N.I. Ceresan	49	58	64	60	47	40	48	52	16 ± 2.9	1: 90
Spergon	54	51	56	51	43	43	45	49	13 ± 2.3	1: 999
Sulfur	26	38	37	37	31	34	31	33	-3 ± 1.3	1:
Leoti sorgo										
Check	59	69	65	62	56	63	48	60	************	***************************************
Arasan	83	87	88	84	90	91	93	88	28 ± 3.2	*
Copper									-	
carbonate	77	79	80	75	76	82	76	78	18 ± 2.0	*
N.I. Ceresan	82	73	59	54	53	70	68	66	6 ± 4.3	1: 6
Spergon	74	79	77	63	60	72	53	68	8 ± 1.7	1: 408
Sulfur	60	67	65	60	54	58	43	58	-2 ± 0.8	1: 39
Norkan sorgo										
Check	49	50	62	55	56	70	54	57	,	
Arasan	72	88	91	86	94	96	93	89	32 ± 2.2	*
Copper										
carbonate	63	-75	79	77	81	87	88	79	22 ± 2.4	*
N.I. Ceresan	48	66	74	72	66	78	80	69	12 ± 2.9	1: 255
Spergon	51	68	74	66	69	82	62	67	10 ± 1.7	1:2499
Sulfur	49	42	59	53	57	57	51	53	-4 ± 1.7	1: 25
Sharon kafir										
Check	56	59	64	61	54	68	52	59	***************************************	
Arasan	58	80	88	87	87	85	95	83	24 ± 5.0	1: 434
Copper								- 00		1. 10.
carbonate	69	80	80	77	76	76	79	77	18 ± 2.2	*
N.I. Ceresan	61	58	66	62	55	71	80	65	6 ± 4.6	1: 6
Spergon	59	58	72	75	56	69	57	64	5 ± 1.8	1: 38
Sulfur	59	61	67	62	56	63	43	58	-1 ± 1.6	1:
Westland mile										
Check	18	34	37	46	36	35	31	34		
Arasan	56	85	90	87	91	88	92	84	50 ± 2.8	*
Copper						-			20 _ 2.0	
carbonate		58	68	70	63	61	56	62	28 ± 1.7	*
N.I. Ceresan	47	57	64	62	49	58	55	56	22 ± 2.1	*
Spergon		38	44	55	40	44	38	40	6 ± 1.0	*
Sulfur	16	32	38	49	35	32	24	32	-2 ± 0.8	1: 15
All varieties										
Check	45	51	54	52	46	55	44	50		
Arasan		85	88	85	89	88	92	85	35 ± 6.6	1: 908
Copper					. 77		~~		20 _ 0.0	1. 300
carbonate		70	75	74	72	74	71	71	21 ± 1.2	*
N.I. Ceresan		62	65	62	54	63	66	61	11 + 1.7	1: 2499
Spergon		59	65	62	54	62	51	58	8 ± 0.6	*
Sulfur	42	48	53	52	47	49	38	47	-3 ± 1.0	1: 39

 $^{^{\}rm a}$ New Improved Ceresan was applied at $^{\rm 1}_2$ ounce per bushel; the others at 2 ounces. *Odds greater than 1: 9999.

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TABLE 6.—Effect of seed treatment and subsequent storage of seed on emergence at 20° C. in sorghum planted in steamed soil

·		E	merge	ence at	ter st	orage at					
Variety and treatment ^a -		20	0° C. f	or		10° C. for	30° C.	Aver- age emer-	Differ- ence	Odds	
	$_{ m days}^2$	20 days	$^{40}_{ m days}$	80 days	$^{180}_{\rm days}$	180 days	180 days	gence			
G1 1 1 C	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.			
Club kafir Check Arasan Copper	63 78	64 87	69 85	71 87	56 85	65 88	62 90	64 86	22 ± 2.0	*	
carbonate N.I. Ceresan Spergon Sulfur		74 85 73 73	81 66 69 66	74 75 74 69	67 64 65 54	80 79 81 65	76 76 73 63	75 75 72 65	11 ± 1.4 11 ± 1.8 8 ± 1.9 1 ± 1.4	1: 333 1: 90 1: 25	
Leoti sorgo CheckArasan		80 87	86 92	75 91	56 86	77 90	$\frac{73}{92}$	74 90	16 ± 2.8	1: 76	
Copper carbonate N.I. Ceresan Spergon Sulfur	78 84	83 69 87 84	83 56 84 77	77 49 78 73	72 50 64 59	79 62 85 77	80 72 71 59	79 62 79 72	$ 5 \pm 2.1 -12 \pm 4.5 5 \pm 1.1 -2 \pm 2.4 $	1: 1: 3: 1: 25:	
Norkan sorgo Check Arasan		82 79	83 91	85 93	72 94	81 95	84 99	81 91	10 ± 2.9	1: 9	
Copper carbonate N.I. Ceresan Spergon Sulfur	74 73	77 71 80 80	88 72 83 88	91 73 90 87	84 67 83 68	88 78 89 79	89 84 90 74	85 74 84 79	$\begin{array}{c} 4 \pm 1.9 \\ - 7 \pm 1.6 \\ 3 \pm 2.2 \\ - 2 \pm 1.6 \end{array}$	1: 28 1: 28 1:	
Sharon kafir Check Arasan Copper		78 73	81 80	80 84	76 90	79 88	69 88	77 82	5 ± 3.1	1: 1	
carbonate N.I. Ceresan Spergon Sulfur	63 69	61 68 71 76	82 60 68 75	83 51 75 72	81 60 74 73	84 71 88 79	84 74 78 69	77 64 75 74	$\begin{array}{c} 0 \pm 3.5 \\ -13 \pm 3.7 \\ -2 \pm 2.9 \\ -3 \pm 1.1 \end{array}$	1: 1: 10 1: 1: 5	
Westland milo Check Arasan Copper		81 71	64 88	66 90	55 93	71 92	60 91	66 87	21 ± 5.3	1: 19	
carbonate N.I. Ceresan Spergon Sulfur	73 68	67 65 71 78	79 61 61 71	78 63 68 63	78 60 66 61	78 69 77 67	79 73 73 63	76 66 69 67	10 ± 4.2 0 ± 3.5 3 ± 2.8 1 ± 1.6	1: 3 1: 1: 1:	
All varieties CheckArasan		77 79	77 87	75 89	63 90	75 91	$\begin{array}{c} 70 \\ 92 \end{array}$	72 87	15 ± 2.9	1: 60	
carbonate N.I. Ceresan Spergon Sulfur	. 73 . 72	72 72 76 78	83 63 73 75	81 62 77 73	76 60 70 63	82 72 84 73	82 76 77 66	79 68 76 71	$ 7 \pm 2.1 \\ - 4 \pm 2.6 \\ 4 \pm 1.8 \\ - 1 \pm 0.7 $	1: 6 1: 1: 1	

See footnote a, table 5.Odds greater than 1: 9999.

at ½ ounce per bushel and with Arasan, copper carbonate, Spergon, and sulfur at 2 ounces per bushel. Each portion of treated seed, contained in a cloth bag, was buried in a half-bushel of seed similarly treated with the same respective chemical and stored at 20° C. and about 70 per cent relative humidity for 2, 20, 40, 80, and 180 days and then germinated. Samples from each portion of treated and untreated seed stored also at 10° C. and at 30° C., and at low relative humidity were included in the germination tests 180 days after treatment. Germination tests were at 20° C. in sterilized and unsterilized compost soil adjusted to 50 per cent of its water-holding capacity, and stored in large metal cans with dust-tight covers until used. The results from treated and untreated seed were analyzed according to Student's method and are presented in tables 5 and 6.

In the tests in unsterilized soil, there were no significant decreases in emergence from treated seed as compared with that from untreated seed except in the case of sulfur. With few exceptions, the four other fungicides apparently caused highly significant increases in emergence, the greatest, by far, being due to Arasan. Emergence from Arasan-treated seed of all 5 varieties was lower in the test planted 2 days after treatment than in subsequent tests, indicating the advisability of treatment with this fungicide at least several weeks before planting. The length of the storage period after treatment did not consistently reduce emergence from treated seed planted in unsterilized soil except, to some extent, in the case of Leoti treated with N.I. Ceresan. There were no significant differences between the percentages of emergence from seed stored at 10° C. and at 30° C. for 180 days after treatment with Arasan, copper carbonate, or N.I. Ceresan; but emergence from untreated seed and from seed treated with Spergon or sulfur was significantly better after storage at 10° C. than at 30° C.

In the tests in steamed soil, in which the harmful effects of certain soil-borne fungi were eliminated, increases in emergence, due to the fungicides, were fewer and smaller, while decreases were more numerous and greater than in unsterilized soil. Arasan improved emergence significantly in 4 varieties, copper carbonate in 3 varieties, and Spergon in 2 varieties. N.I. Ceresan improved emergence significantly in Club kafir and decreased it significantly in Leoti and Norkan sorgos and Sharon kafir. Sulfur showed no significant beneficial effects, and in 2 varieties seemed to cause small decreases one of which approached the level of significance.

It seems apparent that clean seed of good quality, of low moisture content, and free from injury, does not lose its vitality any more readily when properly dusted with any of the better fungicides and properly stored, than does untreated seed, except in the case of certain volatile mercury compounds such as N.I. Ceresan and DuBay 1452–C that injure seed of some varieties. Under unfavorable soil conditions this injury may not be apparent because of being outweighed by the protective effect of the fungicide against soil-borne fungi.

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SUMMARY

Emergence in Sharon kafir in field experiments in 1943 was significantly improved following seed treatment with N.I. Ceresan, copper carbonate, Spergon, Arasan, DuBay 1452–C, Leytosan, and morpholine thiuramdisulfide. In Leoti sorgo emergence was significantly increased by copper carbonate (2 ounces), Arasan (1 and 2 ounces), and by DuBay 1452–C (½ ounce). It was significantly reduced by N.I. Ceresan (½ ounce), DuBay 1452–C (½ ounce), Merc-o-dust, and sulfur. Similar increases were obtained in 1944 with N.I. Ceresan, copper carbonate, Arasan, Spergon, U.S.R. 604, Fermate, Zimate, and basic copper sulfate.

With only 22.3 per cent and 13.2 per cent infection in Sharon and Leoti, respectively, all of the 9 dusts used in 1943 controlled covered kernel smut fairly well except Merc-o-dust, which proved entirely ineffective as a fungicide. In 1944, with 61.3 per cent smut in Sharon kafir grown from untreated seed, infection was reduced to less than 1 per cent by N.I. Ceresan (½ ounce), copper carbonate (1 ounce), Spergon (1 ounce), U.S.R. 604 (1 ounce), and basic copper sulfate (2 ounces). Arasan (at 2 ounces per bushel) reduced infection to 1.1 per cent, while Zimate, Fermate, and sulfur were somewhat less effective. Arasan, applied at 1 ounce per bushel failed to control smut satisfactorily in the 1944 tests.

Under conditions favoring severe smut infection (75.7 per cent) only the volatile mercury dusts, DuBay 1452–F ($\frac{1}{2}$ ounce), and N.I. Ceresan ($\frac{1}{2}$ ounce) approached satisfactory smut control in a variety (Scarborough broomcorn) having seed with persistent glumes, while copper carbonate (3 ounces), Arasan (1 ounce), and Spergon ($\frac{1}{2}$ ounce), allowed 31.7, 39.3, and 48.2 per cent smut, respectively.

Treated and untreated seed of 5 varieties was stored at 20° C. and 70 per cent relative humidity for 180 days. In germination tests at 20° C. in steamed soil after 2, 20, 40, 80, and 180 days' storage, Arasan, copper carbonate, and Spergon either improved emergence or did not impair it, while N.I. Ceresan reduced emergence significantly in 3 varieties, and improved it in one. In unsterilized soil the protective effect of N.I. Ceresan, in general, outweighed its harmful effects, while the other three fungicides improved emergence significantly. Sulfur caused no improvement and some injury.

BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND
AND

NEBRASKA AGRICULTURAL EXPERIMENT STATION, LINCOLN, NEBRASKA.

REPORT OF THE 1945 ANNUAL MEETING OF THE NEW ENGLAND DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The annual meeting of the New England Division was held at Waltham, Massachusetts, February 15 and 16, in conjunction with the New England Vegetable Growers Conference. Twelve technical papers were presented in morning and afternoon sessions on Feb. 15, and a business meeting was held in the evening. A round table discussion on vegetable disease problems and control recommendations took place on Feb. 16.

Officers of the Division for 1945 are: President, Donald Folsom, Maine Agricultural Experiment Station, Orono; Vice President, F. L. Howard, Rhode Island State College, Kingston; Secretary-Treasurer, Thomas Sproston, Jr., Massachusetts State College, Amherst; and Councilor, M. C. Richards, University of New Hampshire, Durham.

ABSTRACTS OF PAPERS PRESENTED AT THE ANNUAL MEETING OF THE NEW ENGLAND DIVISION

Intrascasonal advance of disease to evaluate fungicides or genetical differences. Barratt, R. W. The rate of progress of a disease during the growing season has been found valuable in studying responses to fungicides and other variables. The technique of using this rate of progression involves disease readings by a grading system on the individual plants in each plot at regular intervals over the growing season. The average disease in per cent for each plot at each reading is calculated and graphed on arithmetic-probability coordinates to give the disease trend curve during the season. From the trend curve, which often approximates a straight line, the number of days necessary for a variable to reach any given level of disease can be ascertained by interpolation. This technique has been used in evaluating the field performance of fungicides and tomato varieties. When several concentrations of a material are to be compared, the disease delay at the desired level for each dose is first obtained. Then disease delay in days is plotted against concentration on arithmetic coordinates. Such a curve permits the simultaneous evaluation of three variables—time, concentration, and response. The technique has been adapted to the laboratory assay of the rate of loss in potency of a fungicide.

Control of onion smut by fungicides applied to the soil. Doran, W. L., and T. Sproston, Jr. Onion smut, caused by Urocystis cepulae, was well controlled in greenhouse experiments by Fernate mixed with 5-8-7 fertilizer and applied to the soil immediately before seeding at the rate, per acre, of 58 pounds Fermate in 1500 pounds of fertilizer. Fertilizer alone lessened the severity of smut. Thus, in a typical instance, the percentages of seedlings that became infected were 88 per cent in untreated soil, 56 per cent in soil with fertilizer, and 1 per cent in soil with Fermate and fertilizer. Fermate either improved the growth of seedlings or was without effect upon it. Arasan similarly used gave comparable results. There was good control of the disease with Puratized N5-X and with the nitrites of sodium and calcium, but Fermate gave better results. There was fair control with urea and with calcium cyanamide but, as used, they caused some injury, as did also potassium dichromate.

Polymodal response curves in biological research. Gries, G. A., J. G. Horsfall, and N. Turner. The polymodal curve represents a series of responses distributed in two or more modes. This phrase is preferred to the commonly used periodic or cyclic response curves which connote time. Such curves are apparently generated by the interaction of two dose factors or elements in the stimulus that effect the response when studied over a sufficiently wide range of ratios. They have been observed in research: (1) in the balance between calcium and potassium on the development of potato scab and club root of eabbage; (2) in the effect of pyrophyllite on the insecticidal potency of derris; (3) in the fungistatic dosage-response curves of tetramethylthiuramdisulphide; (4) in the combined effect of sulphur and copper on Sclerotinia. Numerous others have been found in the literature as, for example, in the effect of the balance between H+ and OH- on fungus growth, and between salts on the growth of plants. These curves are very similar to those

obtained with many physical systems, including the Liesegang phenomenon, in which a colloid is involved. The configuration of the curve may suggest the mechanism by which the two factors involved may interact. One such type of interaction which this curve may clarify is in the study of systems in which both antagonism and synergism are displayed.

Studies on the identity and control of stilbaceous mold in gas-purifying sponge. Guba, E. F., and E. V. Seeler, Jr. A phaeo-stilbaceous mold agreeing with the fungus Sporocybe Borzinii G. Goidanich = Petriella Lindforsii Curzi has been found fouling and partly sealing the sponge in the gas-purifying boxes at the Everett (Massachusetts) plant of the Boston Consolidated Gas Company. The gas-purifying sponge employed in the removal of hydrogen sulphide is prepared by mixing 26 pounds of bauxite residue (a by-product obtained in the extraction of aluminum oxide from its ore) or ground bog ore, each essentially iron oxide, with each bushel of wood shavings. Each purifying box contains approximately 10,000 bushels of sponge. The fungus has been reported previously from Europe, in wood pulp destined for paper manufacture and in decaying wood in leaf mold. A phaeo-stilbaceous mold similar to Graphium spp. was found in the dry box purification systems of gas-producing plants in Washington, Philadelphia, and other nearby places in 1938 and 1939. The injection of steam into the sponge and purification structure, and its confinement to obtain a uniform lethal temperature, is recommended for control. Temperatures of 180° F. for 15 minutes, intimate with the fungus, are lethal. An initial highly alkaline reserve in the sponge, and the maintenance of alkalinity upwards of pH 10.0, is repressive to the fungus. The funigation of the sponge in situ with formaldehyde is recommended. Wetting down the top sponge layer in the boxes with formaldehyde diluted 1–50 or with 0.2 per cent sodium penta-chlorphenate solution, or any other suitable disinfectant, is suggested. Sterilization of the wood shavings or readymade sponge with heat or chemicals before installing the boxes is desirable.

An improved grading system for measuring plant diseases. Horsfall, J. G., and R. W. Barratt. Heretofore, in recording severity of plant diseases by grading, the grades have been assigned equal values in percentage. According to the Weber-Fechner law, the human eye distinguishes according to the logarithm of the light intensity. Hence, the grades should be based on equal ability to distinguish, not on equal disease. Below 50 per cent, the eye sees the amount of diseased tissue. Above 50 per cent, it sees the amount of disease-free tissue. A new scoring system is based on 50 per cent as a midpoint. The grades differ by a factor of two in either direction as follows: 1=0, 2=0 to 3, 3=3 to 6, 4=6 to 12, 5=12 to 25, 6=25 to 50, 7=50 to 75, 8=75 to 87, 9=87 to 94, 10=94 to 97, 11=97 to 100, 12=100. Several plants (20 or more) at random are graded. Mean grade = grade readings + number of readings. A calibration curve is set up with grade numbers on the X-axis and percentage disease on a special semi-log. Y-axis with one and one-half phases from either end up to 50 per cent. The grid has the aspect of arithmetic-probability paper. This scheme has been very useful in fungicide research, varietal resistance, etc. It should be useful in plant disease surveying.

Susceptibility of Logan and Florida Belle beans to Fusarium yellows. Howard, F. L., and E. M. Andersen. Characteristic Fusarium yellows developed in Logan and Florida Belle among eleven snap bean varieties (Tendergreen, Asgrow Stringless Green Pod, Asgrow Black Valentine, Burpee's Stringless Green Pod, Bountiful, Plentiful, Pencil Pod Black Wax, Surecrop Stringless Wax, Keystonian, Logan, and two lots of Florida Belle) planted in a randomized, four-replicate design at the Rhode Island Experiment Station in 1944. Following a dry summer with high soil temperatures 37 per cent and 45 per cent of the Florida Belle and 82 per cent of the Logan foliage was dead at the close of the picking season on September 1. None of the other varieties had such disease symptoms. This experience emphasizes the necessity of trying new varieties under local edaphic conditions before extensive plantings are made.

Factors influencing the development of resistant sporangia on Allomyces arbusculus. Jones, R. C. Experiments were carried out to determine the possible roles of light and temperature in limiting the number of resistant sporangia (structure in which meiosis normally occurs) formed in cultures of Allomyces arbusculus grown on maltose-peptone agar. Results suggested that the frequent failure of Allomyces to develop resistant sporangia during the summer could not be attributed to the longer photoperiod of that season. In cultures grown at relatively high temperatures, resistant sporangia were entirely lacking, or were formed only in limited numbers. Further experimentation showed that high aggregate temperatures were more limiting than were high maximum temperatures. Within the temperature limits of these experiments, the number of resistant sporangia per given area became successively smaller as the number of degree hours of temperature per day was increased, even when the same minimum and maximum temperatures were maintained.

Investigation of the Dutch elm disease in Massachusetts. McKenzie, M. A. Since 1941, when the first tree in Massachusetts known to be affected by the Dutch elm disease was found at Alford, 32 additional affected trees have been found nearby in Berkshire County and 10 diseased trees in Hampden County. Some factors may help and some factors may handicap control. Handicaps include: 1. Large numbers of "weed" elms near interstate highways, railroads, and rivers. 2. Prevailing winds in relation to spread of principal carrier beetle. 3. Elm in woodpiles. 4. Hurricanes, ice storms, and high winds followed by increase in material for beetle breeding. 5. Conditions favorable for spread of disease in areas adjacent to Connecticut River. Factors that may help in the control of the disease include: 1. Elms sometimes in mixed plantings on streets. 2. Some elms well maintained. 3. Possible climatic influences. 4. Assistance of every agency whose work relates to trees. 5. Lower rate of increase in number of diseased trees in Massachusetts during first 3 years than in many areas elsewhere for a corresponding period. 6. Cooperative program of U. S. Department of Agriculture, State Department of Agriculture, and Massachusetts Agricultural Experiment Station for collecting data on diseased trees, carrying out sanitation and other control measures, and conducting experiments.

The control of Alternaria blight on N. H. Victor tomatoes by the application of fungicides. RICHARDS, M. C. New Hampshire Victor tomato plants set 5×5 ft. in the field were sprayed five times from July 20 to August 26, 1944, with three materials: Copper Oxychloride Sulphate 1, 2, 4, and 8 pounds per 100; Bordeaux Mixture 1-1-, 2-2-, 4-4-, and 8-8-100; and Fermate ½, 1, 2, and 4 pounds per 100. Each of the 12 treatments resulted in highly significant increases in yields of marketable fruits. None of the treatments increased the total yields significantly. The highest yield of marketable fruit was obtained with the Fermate 4-100, a yield of 19.8 pounds per plant as compared to 9.4 pounds for the check. Equivalent control at 88.0 per cent defoliation was obtained with the following: Fermate 1-100, Bordeaux Mixture 4-4-100, and Copper Oxychloride Sulphate 9.5-100.

Changes in the skin of the Blue Hubbard squash during storage and its relation to spoilage of the squash fruits in storage. RICHARDS, M. C., A. F. YEAGER, and R. C. JONES. Disease losses in storage on Blue Hubbard squash were materially reduced when the fruits were dried by using heat for the first two weeks of the storage period. Drying of the skin decreased the number of infections through minor cuts and bruises made during the harvesting operations. No noticeable morphological changes took place in the cells of the skin during the 14-day "curing" period. In the Blue Hubbard squash variety, where both thick and thin-skinned types occur, there was gradual lignification of the collenchyma cells during the 5-month storage, as shown by the phloroglucin test. Slight lignification was found in the mature, thick-skinned types at harvest time. After four months' storage, both the thick- and the thin-skinned types, showing heavy lignification, spoiled from numerous infections by Alternaria, Fusarium, and Botrytis when the fruits were placed under conditions favorable for infection in a cold storage room at 35-40° F. A dry room at 50-60° F., following the two weeks curing period, was most satisfactory for storage. Removing both mature and immature fruits, before frost, directly from the field to the storage and removing the stems materially reduced losses from black rot.

Onion seed treatments. Sproston, T., Jr., and O. C. Boyd. Seed treatments were used for onion and set production. The trials on seed for big onions included 8 treatments replicated 5 times in a latin-square designed experiment: Arasan, Thiosan, and Fermate (100 per cent and 75 per cent by weight of seed), Formaldehyde 1–100 at 100 gal. per acre, and cheek. Data on total stand of seedlings and smutted seedlings were subjected to analysis of variance. On total stand of plants counted three weeks after sowing, all dry treatments stuck on the seed with methocal were significant (5 per cent point) over Formaldehyde and check. Arasan 100 per cent. Using mean numbers of smutted seedlings, all treatments except Arasan 75 per cent. Using mean numbers of smutted seedlings, all treatments were significant (1 per cent and 5 per cent points) over the check. However, there were no significant differences between any of the treatments in the control of smut. Data obtained in Massachusetts during 1943 and 1944 favor the use of dry stuck-on seed treatments, preferably Arasan 100 per cent or 75 per cent, because of increased total stands of seedlings and not because of smut control alone. For set production there were twenty treatments: Arasan, Fermate, and Thiosan at dosage rates by weight of seed of 2 and 5 per cent not stuck on seed (-), and at rate of 2, 5, 10, 15, and 25 per cent stuck on (+); Formaldehyde 1–60 at 100 gal. per acre; and no treatment. Arasan, stuck on or dry, was significant (5 per cent point) in total stand of plants over all other treatments per dosage rate groups. Percentages of smutted seedlings were as follows: checks 35.7, Arasan 15 per cent (+) 1.7, Arasan 5 per cent (-) 3.07, Arasan 2 per cent (-) 7.9, Formaldehyde 8.3. Sets were graded into 3 classes \(\frac{1-3}{2}, \frac{1}{2-4}, \frac{7}{4}-1 inch.

All treatments including checks produced about 70 per cent good sets (½-¼ inch). Considering ease of operation and results of set production, Arasan 5 per cent (-) to 8 per cent (-) would be preferable to Formaldehyde.

Red spider control with disodium ethylene bisdithiocarbamate. Stoddard, E. M., G. A. Gries, and G. H. Plumb. In greenhouse experiments considerable success was obtained in the control of the common red spider or red mite (Tetranychus bimaculatus) by spraying with disodium ethylene bisdithiocarbamate (Dithane). When used with 0.00125 per cent B-1956 spreader, the adults and nymphs of this pest were killed by all concentrations above 0.125 per cent Dithane and most of those in the egg stage were killed by a spray of 0.6 per cent. A spray of 0.15 per cent Dithane plus the spreader, repeated after ten days, controlled red spiders on strawberries and earnations. Spray injury was not severe below approximately 0.5 per cent on strawberries and peaches, but some discoloration of carnation foliage occurred at a concentration of 0.15 per cent. Experiments in which the spreader was omitted consistently showed less injury to the plants and a lower kill of the mite population. The spruce mite, Paratetranychus nununguis, was controlled by immersing the cut ends of spruce twigs in a 0.6 per cent solution of Dithane. This indicated that control of this species was effected by the absorption of the chemical by the twigs. No population reduction was observed on check twigs immersed in water. The common red mite was not controlled when cut petioles of strawberries were immersed in various concentrations or when the solutions were added to the soil.

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HERBERT HICE WHETZEL 1877–1944

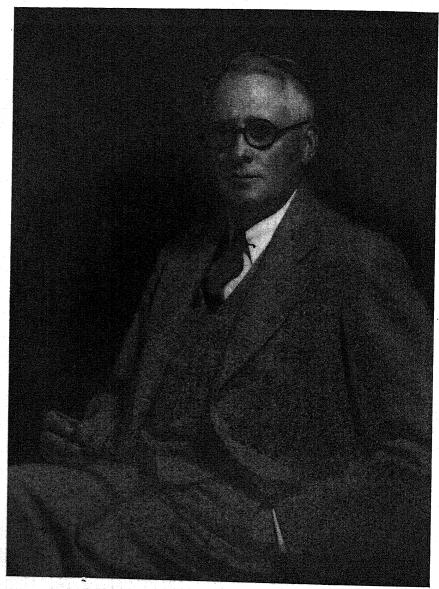
M. F. BARRUS AND E. C. STAKMAN

The value of many a man's life may be judged most fairly by his influence on others. This intangible attribute is known fully only by those who have felt it. Professor Whetzel had a profound influence on many persons as is evident from the affection they have had for him, the testimonies they have offered, and their activity in carrying forward his suggestions. He was a unique character with extraordinary talents, some of them at least closely akin to genius. He was a great teacher, an enthusiastic naturalist, a keen investigator, an efficient organizer, a scientific leader, and a dynamic man. This is the composite judgment of his students, his colleagues, and many of his professional acquaintances. It is the judgment not merely of his friends but also of those who have considered his contributions with detached objectivity, apart from the immediate influence of his powerful personality. It is simple justice to record the debt that American phytopathology and phytopathologists owe to this indefatigable champion of his chosen scientific guild.

Herbert Hice Whetzel died at his home on November 30, 1944, after being confined to his bed for three weeks by a disease against which he had valiantly fought during a period of ten years. Yet, despite the inconvenience and pain it caused him, he continued his work until nearly the end of his life.

Whetzel was born on a farm near Avilla, Indiana, on September 5, 1877, and obtained his early education at a rural school and at Avilla High School. After a period of teaching district schools, he entered Wabash College from which he graduated in 1902. While there, he came under the influence of Professor Mason B. Thomas, a Cornell man, and a most excellent teacher of botany. Probably it was due to this influence that Whetzel majored in botany. It was Thomas who obtained for him an assistantship at Cornell University and an opportunity to pursue graduate work under another great botanist, Professor George F. Atkinson. He later became an instructor in botany at Cornell and most of his time was devoted to the study of plant Before he could complete his work for a doctorate, Dean Liberty Hyde Bailey, recognizing his talents, appointed him, in 1906, an assistant professor and head of a new department of botany in the New York State College of Agriculture to be devoted to teaching and research work in plant The following year, at the earnest request of Whetzel, the name was changed to department of plant pathology. He was appointed professor in 1909 and retained this title throughout his life, although he resigned the headship of the department after an interval of sixteen years in order to devote his full time to teaching and research. He was convinced also that, with rare exceptions, no man should be allowed to head a department for

¹ Professor H. M. Fitzpatrick has prepared a personal account of Professor Whetzel's life to be published in "Mycologia" during 1945.



HERBERT HICE WHETZEL 1877-1944

more than fifteen years because long tenure deprives ambitious and able young men of the staff of an opportunity to do administrative work and he thought it might be injurious to the department or the institution itself.²

Professor Whetzel was first of all a teacher. His profound knowledge of phytopathology, his enthusiasm, and his interest in those willing to work attracted students to him. He had an unusual ability to present his subject clearly and logically. He taught the elementary courses himself because he believed the undergraduate students needed the clear presentation of the subject and the inspiration he was able to give them. This also gave him the opportunity to discover exceptionally gifted students and to persuade them to take graduate work. In his early years of teaching, he spent much time in preparing reading and laboratory texts of each disease used for class study, according to an outline he had originated. These were revised from time to time with the help of his colleagues in the department and two editions of the laboratory text were later published.

Whetzel took the attitude that students who registered for his courses did so because they wanted to obtain a knowledge of the subject. No record was kept of their attendance at lectures and laboratory periods; they could come or not as they wished. In order to obtain credit in a course, however, they must complete satisfactorily the work assigned. Being dissatisfied with the usual method of teaching, he devised a system whereby students select for study, within groups, certain diseases in which they are especially interested. They are provided with ample material and with the laboratory and reading texts. They may work on any disease as long as they wish, for the laboratories are always open. After completing the study of a disease, they are given an individual conference with an instructor at which the student demonstrates his knowledge of the subject by the way he uses it in solving hypothetical problems.

The graduate students, however, were not neglected. He was always available to them and his fertile mind was full of ideas relating to their problems. He was quick to give praise for their accomplishments and willing to give them full credit for their discoveries even when his suggestions made them possible. That they might know the background of their science, he published an outline of the history of phytopathology. He also gave a course in scientific German reading, using a method he devised which enabled the student to acquire a proficiency in reading the language within a year. He did whatever was possible to fit them for their profession and they left for their own fields of activity with a deep sense of gratitude and an enduring attachment to him.

But Whetzel's influence extended far beyond his own classroom. During the period of his greatest vigor, he made a two-fisted fight for better teaching and he expounded his pedagogical ideas far and wide with a zealousness born of intense conviction. To him the individual student was the most important element in education. He often said "Nobody ever

² Jones, L. R. Whetzel resigns headship—deplores present system of administrative tenure. Phytopath. 12: 499-500. 1922.

taught anybody anything." His idea was that a teacher was good not so much because of what he transmitted to students but because of what he got them to learn. This educational doctrine was not new but Whetzel really made it work. Students who had passed his individual-effort courses stuck out their chests a little farther and elevated their chins a little higher: they had acquired confidence in their own intellectual power, in their ability to find out about things by themselves. Insistence on individual work, on respect for facts, on refinement of concepts, on precise terms and definitions, and on organization of knowledge was the keystone of Whetzel's pedagogical creed. And, born agitator that he was, Whetzel at least jarred many other teachers out of complacency and, in his peppery way, injected enough intellectual irritants into their minds to make them think about improving their own methods even if they did not accept his.

Whetzel always wanted farmers to have the best and latest information on disease control. In the early years of the department's life, he spent considerable time in preparing exhibits and in attending fairs. He addressed farmers many times at state and county meetings and used every opportunity to impress upon them the importance of controlling diseases. Once, he gave a talk on apple scab, using charts, at a night meeting of the state horticultural society. After the meeting, the extension director who is also a fruit grower, remarked, "If anyone had told me I would sit on a hard bench in a hot crowded room for two hours listening to a man spout about apple scab, I would have told him he was crazy. But I did and I liked it and I learned a lot." During those years, Whetzel was well known and well liked by New York farmers. They had confidence in him even to the extent of being willing to contribute money for research work on their disease problems when he proposed it.

He believed in the socialization of his science. But he was hard-headed enough to maintain that plant growers would appreciate and use most effectively those services for which they paid in hard cash. He was confident that eventually even the private plant doctor would find a field of usefulness. At a time when many agricultural scientists still looked askance at funds offered by commercial organizations for research, Whetzel boldly encouraged and advocated the industrial fellowship plan and made it work. Not only corporations but also growers' organizations were brought into partnership with the University in the investigation of plant-disease problems. These fellowships made it possible for qualified students to undertake graduate work who would have been unable to do so without financial assistance and they have resulted in the accomplishment of much practical experimental work that, without them, would not have been done.

The generation of plant pathologists who were in process of professional development while Whetzel was in his prime probably were most impressed by his Kühnian attitude: the utilization of basic information in plant-disease control. Certainly Whetzel and his associates at Cornell were in the vanguard of those pioneers who began to emancipate practical plant pathology

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from a murky empiricism and place it on a sound scientific basis. He appreciated the possibilities of improvement in control methods and materials. As soon as he learned of Cordley's results with lime-sulphur as a fungicide, he had a fellowship organized to study further the possibilities of this material in controlling diseases in New York State. He championed dusting as a substitute for spraying in the early days of its use for disease control and defended the method in the face of severe criticism when its limitations became apparent, for he believed that, with improvement in quality of dusts and in machinery for applying it, this method of protection would be generally used.

Despite his advocacy of exacting standards of experimentation in a very practical field of endeavor, Whetzel remained at heart a naturalist, a collector, and a mycologist. His intimate acquaintanceship with growing things in the woods and fields was not only the envy but also the despair of many who had the desire but not the ability to master successfully so wide a range of pure and applied mycology.

During his later years, he devoted much time to the study of the Sclerotiniaceae in which he was interested throughout most of his professional life. He collected assiduously every spring and also received specimens from other mycologists knowing his interest in this group. Many papers relating to the genera and species have been published by him and by his students. At one time he planned to prepare a monograph of the North American species but, as his work progressed, he recognized the need for a more detailed study of the new species discovered and for erecting new genera before it would be possible to monograph them. Realizing finally that his time was short, he started to prepare a characterization of the new family Sclerotiniaceae and a synopsis of the fifteen genera he had included in it. Although it is unfortunate that he was unable to complete this before his death, he did succeed in greatly increasing our knowledge of this important family of fungi.

Whetzel's researches cover a wide range in phytopathology, as the list of his publications indicates. While he was still an instructor in botany, he published three important bulletins on plant-disease control. During his lifetime he was active in studying diseases of many kinds of plants, including medicinal, ornamental, and wild plants, and in the study of fungicides and control measures. And yet to many pathologists Whetzel's critical faculty may have been even more stimulating than his own researches. It is a great misfortune that he published so little of his vast store of information, but his "Are you sure of that?"; his "I'll bet you a cigar that I can prove you wrong"; and his less contentious "Did you ever try this—?" in meetings, in his favorite "rag-chewing sessions," and in correspondence suggested many a new line of attack on obscure problems and led to many a discovery. Dynamic he was, not only in teaching but also in research, and he added to his zeal for both a profound faith in the service of organized plant pathology in plant production and plant protection.

The plant pathology herbarium at Cornell University is a monument to Whetzel's interest in fungi. His own collections formed its nucleus to which he and others have added many specimens since. It now contains more than 31,000 specimens besides private and special collections and purchased exsicatae. Since 1922, when he first collected in Bermuda, he had been active in building up collections from the Tropics. He made three trips to Puerto Rico and one to Venezuela during which he collected extensively with other mycologists. He collected fungi wherever he went and stimulated his students to do likewise, with the result that specimens have come from many countries and especially from Latin-America.

Whetzel was one of the charter members who organized the American Phytopathological Society in 1909 and was one of its guiding spirits until his physical condition prevented him from attending meetings. And he worked hard to make the Society useful not only to its members but to the advancement of science as well. He was one of the promoters in the organization of the Mycological Society of America and took a leading part in its affairs as well as contributed to its official journal.

Gardening was another activity that gave him much pleasure. He grew many kinds of plants, including new varieties of vegetables and fruits. His greatest interest was wild and cultivated varieties of flowering plants, especially unusual and rare species and varieties. He delighted to show these to the many persons attracted to his garden and generously gave plants and seeds to those who indicated an interest in them. From this garden also came many prized specimens of plant diseases he had discovered and maintained.

A many-sided man, Whetzel was an intensely interesting character. It is difficult to suppress an impulse to write about his human qualities because, to those who knew him best, his sterling character and his capacity for enduring friendship command respect and admiration quite as much as his intellectual contributions. His friends will always keep something of "Whet" within themselves. But this is being written not as a eulogy but as an evaluation of Whetzel's services to Plant Pathology. To say that he was "Prof" to many aspiring young pathologists who never entered his classroom would alone be high tribute. But, it is even higher tribute to say that he was still "Prof" to many of his professional associates who fought him hard on certain issues. For "Prof" was a rugged, aggressive, and game fighter. He fought for his convictions and came into an argument with crackling energy and a determination to rush his opponents through the ropes. He never held resentment long against anyone and never spoke maliciously of others.

No longer will his footsteps be heard in the corridors of the University nor his cheery "Howdy" greet his friends as in the past. His voice will not be raised in arguments at gatherings he loved to attend nor will his friendly counsel guide his students anymore. Yet, in the hearts of all who knew him will remain the memory of the man, and his influence will prevail for many years to come. His life was full and rich because he lived it with others.

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STRUMELLA CANKER OF OAK

BAILEY SLEETH AND ROLLAND C. LORENZI (Accepted for publication March 26, 1945)

The distribution, host species, and symptoms of the canker disease attributed to the fungus Strumella coryneoidea Sacc. have been described by Heald and Studhalter² and Bidwell and Bramble.³ These investigators, on the basis of association, determined the causal fungus to be Strumella coryneoidea Sacc. Heald and Studhalter state that they secured successful inoculations in the field. However, they do not indicate the source of the cultures or the methods used, or give data on number of successful inoculations or illustrations of induced infections. Because of the lack of definite information concerning inoculations and the production of infection and cankers, it was considered advisable in the course of general work on control of the Strumella canker disease to inoculate young oak trees with the fungus, which had been consistently isolated from Strumella cankers, in an attempt to establish infection and produce typical cankers.

INOCULATIONS

The inoculations herein reported were made in the fall of 1934 in the Logan State Forest in the vicinity of State College, Pa., and the following year in Connecticut. Saplings of the following species were inoculated: White oak (Quercus alba L.), chestnut oak (Q. montana Willd.), red oak (Q. borealis var. maxima (Marsh.) Ashe), scarlet oak (Q. coccinea Muench.), and black oak (Q. velutina Lam.). The trees inoculated ranged from dominant to suppressed types. Most of the inoculations were made with cultures bat had been isolated from typical Strumella cankers from different oaks ble 1), but a few were made with masses of spores from freshly collected ting bodies of Strumella coryneoidea found on dead oak branches.

Live branches \(\frac{1}{4}\) to 3 inches in size were inoculated. Some branches were girdled a month before they were inoculated and some were girdled when inoculated. Inoculations were made either above or below the girdle and in some cases both above and below. The purpose of the girdle was to weaken, but not kill, the branch. It was thought that a branch thus weakened by girdling might provide conditions favorable for the establishment of infection and subsequent canker formation. Also, inoculations were made on ungirdled branches and trunks of very small trees. The inoculations per tree ranged from 3 to 6. The inoculum, other than spores from fruiting bodies, consisted of autoclaved rice on which the fungus had grown.

¹ Formerly Assistant Plant Pathologists, Civilian Conservation Corps, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture; now, respectively, Associate Pathologist, Guayule Emergency Rubber Project, and Senior Plant Pathologist, Office of Foreign Agricultural Relations.

² Heald, F. D., and R. A. Studhalter. The Strumella disease of oak and chestnut trees. Pa. Dept. Forestry Bull. 10. 1914.

³ Bidwell, C. B., and W. C. Bramble. The Strumella disease in southern Connecticut, Jour. Forestry 32: 15-23. 1934.

The technique of inoculation was similar to that described by Hahn and Ayres.⁴ The area around the point to be inoculated was carefully washed with 95 per cent alcohol. A small short-handled chisel, sterilized by dipping in alcohol and flaming, was used to make an incision, approximately 1 inch in length, parallel to the long axis of the trunk or branch and just through the bark. The bark was raised slightly on one side of the slit by pushing the tip of the chisel under the bark and giving a slight pry on the handle of the chisel. In the opening thus made a small quantity of rice inoculum was placed. In a few cases where masses of spores were used the inoculation procedure was essentially the same. A small pad of sterilized cotton was placed over the inoculated wound and bound firmly in place by a cotton

TABLE 1.—Reisolations from Strumella inoculations made at Sand Spring, Logan State Forest, Pennsylvanias

Source of	Oaks inoculated and number of inoculations yielding Strumella when cultured										
cultures -	White	oak	Chestni	ıt oak		Black	coak		Red	oak	
	+b	_b	+	-		+	-		+	_	
White oak	2	0	2	0		2	0		1	0	
White oakc	2	2	1.	1	: 1	1	0		1	0	
Chestnut oak	3	0	2	. 0		1	0		1	0	
Scarlet oak	5	1	1	1		1	0		1	0	
Black oak	2	1	1	1		1	0		1	0	
Cotal	14	4	7	3		6	0		5	0	

^a The Strumella fungus was recovered in all cases in invaded tissue beyond the point of inoculation. All inoculations made with sterile rice inoculum gave negative results.

string. In a few cases in which no cotton was used, the wound was wrapped with the cotton string. The tight binding forcibly brought the inoculum in contact with the ruptured bark tissues. Finally, the wound was carefully wrapped with a 3- to 5-inch band of waxed paper or parchment paper, which was fastened at the top and bottom with commercial zinc oxide adhesive tape, or mechanic's tarred tape. The top of the paper band was bound tightly to the branch or stem with tape. In most cases this made an effective waterproof seal. The wrapping protected the incisions from contaminations and prevented excessive drying of the ruptured and inoculated tissues. Checks were made in a similar manner, in which sterile rice was used for inoculum.

RESULTS

A small number of inoculated branches were removed at various intervals to determine whether the Strumella fungus had become established in the inoculated tissues and to note the progress of infection, if any. In May, 1935, 6 months after inoculation, Strumella was recovered from 12 in-

⁴ Hahn, Glenn Gardner, and Theodore T. Ayers. Failure of Dasyscypha willkommii and related large-spore species to parasitize Dougles fir. Phytopath. 28: 50-57. 1938.

b+indicates infection; - indicates no infection.

This culture was from a pustule on a diffuse Strumella canker. The other cultures used were from cankered tissue of typical "cat-faced" cankers.

oculated branches out of 14 removed for examination and culturing. The checks were negative. The fungus had established itself in the wounded tissue and had advanced a slight distance into the uninjured live tissue adjacent to the incision (Fig. 1, A). These results indicate that the fungus was able to spread to some extent during the winter.

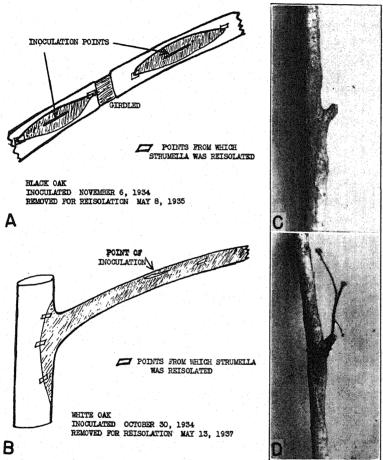


FIG. 1. Development of Strumella cankers on inoculated oaks. (A) Sketch of a girdled black oak branch 6 months after it had been inoculated (shaded portion indicates dead area). (B) Sketch of a white oak branch 2½ years after it had been inoculated. The fungus has progressed down the inoculated branch into the trunk. Strumella canker development 3 years after inoculation, (C) on chestnut oak and (D) on white oak.

A little over a year after the inoculations were made, December, 1935, a few more inoculated branches were removed. Strumella was recovered in 9 cases out of 11 inoculations made from Strumella cultures. The fungus was recovered at considerably greater distances from the point of inoculation than from those examined in May. Whether the fungus progressed at a uniform rate in the uninjured tissues during the 7-month interval, or made rapid progress after cessation of tree growth in late summer is not known. In a number of cases the fungus had killed the inoculated branch by

girdling. As might be expected, the longitudinal advance of the fungus was much greater than its circumferential progress.

To check on the inoculations made with Strumella spores from sporodochia on small dead oak branches 8 inoculated branches were removed in December, 1935. In only one instance was Strumella isolated from the tissue adjacent to the inoculated incision. This may have been a chance infection from some other source. However, if a sufficient number of successful inoculations could be secured by this method it would establish definitely that the spores thus produced in nature do germinate and are a potential source of inoculum for natural infections. Successful germination of Strumella spores has not been reported to the writers' knowledge.

By May, 1937, many of the inoculated branches were showing definite indications of canker formation. In some cases, two definite somewhat concentric callus rings had developed around the point of inoculation. At this time 8 inoculated branches were removed, 5 of which yielded Strumella. The fungus had killed the inoculated branch in most instances and had progressed down the branch and invaded the trunk in some cases (Fig. 1, B). Two small trunk cankers resulting from branch inoculations are shown in figure 1, C and D. It seems most likely that natural trunk cankers develop in a similar manner, in which infection enters the trunk from a small branch killed by the invading fungus. This would account for the occurrence of a dead branch stub in the center of the canker, which tends to develop in an ellipsoidal fashion.

Late in October, 1937, six inoculated branches with definite canker formation were removed. All 6 cankers yielded Strumella in culture.

Results similar to those secured in Pennsylvania were obtained from inoculations that were made in a plot in Connecticut in October, 1935. Three scarlet oak and three red oak branches that had been inoculated with *Strumella* and that had typical lesions were removed from the Connecticut plot in June, 1937. All 6 inoculated branches yielded the fungus upon culturing.

Successful cross inoculations were made on both girdled and ungirdled branches among a few oak species (Table 1) in the Pennsylvania plot. There was no indication that an isolate from one oak species was more virulent than any other. Unfortunately, observations could not be continued for a sufficient length of time to secure conclusive data on this point. However, the results do indicate that the fungus is not a highly specialized parasite.

SUMMARY

Successful inoculations and initial canker development were secured on various oaks with a fungus, identified as *Strumella coryneoidea*, which had been isolated from typical Strumella cankers.

DIVISION OF FOREST PATHOLOGY,

Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration,

UNITED STATES DEPARTMENT OF AGRICULTURE.

FACTORS AFFECTING THE SAPROGENIC ACTIVITIES OF THE DUTCH ELM DISEASE PATHOGEN

L. J. TYLER AND K. G. PARKER

(Accepted for publication April 2, 1945)

The Dutch elm disease fungus (Ceratostomella ulmi (Schwarz) Buisman) lives as a saprogen for a considerable part of its normal life cycle. During this phase the organism is subject to the influence of a number of environmental factors that may greatly affect its existence. Information about the effects of different factors is rather scarce in the literature and so the purpose of this paper is to help elucidate the effects of temperature and moisture on the saprogenic growth and survivability of $C.\ ulmi.$

LITERATURE REVIEW

Ledeboer (4) reported that *Ceratostomella ulmi* grew fairly well on artificial media at 8.5° C.; the optimum temperature was 25° C. and the maximum about 34° C. May (5) found that, for growth on the media used, the minimum temperature was between 5° and 9° C., the optimum between 22° and 27° C., and the maximum about 36° C. He stated, further, that temperatures from 10° to 35° C. had no differential effect on the relative abundance of coremial fruiting structures produced by the fungus.

True and Slowata (9) found that the Dutch elm disease pathogen survived in naturally diseased cut elm wood at least 20 months under some field conditions. In their test, diseased wood fully or partly shaded from the sunlight all or part of the time maintained the organism in a living state longer than did that exposed to more or less constant sunlight and desiccation.

Buisman (2) reported that spores in dried masses withstood a 2-month drying period in Petri dishes at room temperature. Moses and Hoffman (6) exposed beetles ($Scolytus\ multistriatus$), surface-infested with spore masses, to a series of temperatures that ranged from -10° F. (-23.5° C.) to 70° F. (about 21° C.). The fungus survived about 3 months at 70° F. on 60 per cent of the beetles while after $2\frac{1}{2}$ years it was recovered from all the beetles that were exposed to -10° F. May (5) reported 51° C. as the thermal death point for spores in water and 57° C. for those exposed on dry glass rods.

Tyler et al. (10) reported that high air humidity favored infection of small potted elms inoculated by introducing spores into wounds that were made by noninfested adults of Scolytus multistriatus Marsh.

METHODS AND MATERIALS

Six cultural races of *Ceratostomella ulmi*, all pathogenic to the American elm, were used as noted in the following studies. They originated as sector variants in mass isolates from diseased elms and the stock cultures were

started from single spores. Distinct differences in cultural characters such as those noted by Walter (11) existed among the races. Some races produced coremia abundantly when grown on nutrient agar and on elm wood, others produced none, while still others were intermediate in this character.

The nutrient solution commonly used in spore germination tests consisted of a 0.9 per cent solution of maltose (technical grade) in distilled water; it was sterilized by means of a suitable Berkefeld filter and usually tested about pH 6.2.

The artificial, nutrient medium used was potato-dextrose agar. It consisted of the extract obtained by boiling 390 grams of peeled Irish potatoes for 20 minutes in a quantity of distilled water, 20 grams of dextrose, and 18 grams of shredded agar made up to one liter with distilled water. A quantity of this medium sufficient for a given experiment was prepared in one batch and autoclaved for 20 minutes at 15 pounds pressure. The pH concentration of the nutrient agar after autoclaving usually was about 5.8.

Diseased elm wood used in some of the temperature and humidity experiments was obtained from 3- to 4-year-old potted elms that were artificially inoculated with chosen races of the pathogen. Before the disease had killed the trees, branches $\frac{1}{2}$ to 1 inch in diameter were cut from them and wrapped in waxed paper then stored at 5° C. until needed.

Temperature Studies

Spores from coremia¹ approximately 10 to 15 days old were suspended in the maltose solution. Drops of the spore suspension were placed on sterile glass micro-slides in inverted glass moist chambers. The density of the spores in suspension was adjusted so that about 50 to 75 spores were present in the microscopic field as seen with the dry, high-power objective of a compound microscope. Usually the spores were exposed to the desired temperature 10 to 13 hours before counts were made.

Petri dishes containing the solidified nutrient agar were planted by transferring to each a 4-mm.-wide disc cut from a 4-day-old agar culture of *Ceratostomella ulmi*. Planted plates used in the temperature studies were exposed for 8 days and during this time they were kept within sealed glass chambers to prevent excessive moisture changes in the medium.

The effect of temperature on development of coremia on wood from diseased trees was studied by two culture methods. For one, discs of diseased elm wood about ½ inch in diameter by ½ inch thick were cut from stored material and placed on 1.5 per cent non-nutrient agar in Petri dishes. Triplicate plates containing the discs were placed in Stender chambers whose lids were sealed with vaseline. Such chambers prevented excessive desiccation of the cultures held at the higher temperatures. For the other method, discs of wood similar to the above were placed on sterilized, flat cotton lamp wicks that were drawn through ½-inch-bore glass tubing. The tubing was first cut in lengths such as would fit, when supported horizon-

¹ These spores are termed coremiospores in the paper.

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tally, into a Stender chamber 5½ inches in diameter by 2½ inches deep. One end of the wick dipped into sterilized water in a wide-mouth bottle inclosed within the chamber, the other end extended almost to the opposite end of the tube which was loosely plugged with nonabsorbent cotton. The moist wicks kept the moisture content of the wood at the proper level. Before the fungus-invaded discs of wood were placed in the culture chambers the latter were sterilized by dry heat. After the wood discs were placed within, the chambers were sealed with vaseline and placed at the desired temperatures. The cultures were examined periodically by means of a binocular microscope.

Humidity Studies

To study the effect of air humidity on germination, coremiospores were suspended in maltose solution. The spore suspension was sprayed on surface-sterilized glass micro-slides with an atomizer that was sterilized in boiling water. The sprayed slides were allowed to dry for about 3 hours; afterward they were placed on racks in Stender chambers over a series of salt solutions each of which exerted a different vapor pressure. Each chamber contained 4 slides, 2 with the dried spore suspension facing downward and 2 facing upward. All culture chambers in the series were sealed and exposed at 24° C. After exposures of 24 and 48 hours the slides were examined with a compound microscope.

The effect of air humidity on the germination of spores and on the growth of hyphae on elm wood was studied. Discs of elm wood were cut from fungus-free wood, and from diseased wood. The fungus-free wood discs were sprayed with coremiospores suspended in sterile distilled water. Discs of both kinds were air-dried for 3 hours and some of each were placed in each of four desiccators. Humidity of the air in each desiccator was controlled by first passing it through a suitable salt solution and then through the desiccator. Salt solutions in the towers through which the air was passed were changed every 72 hours. The treatment was continued for 14 days after which the discs were carefully examined. The absence of mycelia was considered as proof of the inability of the organism to grow under the conditions imposed.

Survival Studies

The effects of higher temperatures and of moisture on the survivability of *Ceratostomella ulmi* were studied using discs of diseased elm wood like those described. By means of a simple irrigation device, wood discs inclosed in sealed Stender chambers were kept moist but not wet. In similar chambers, without the irrigation device, other wood discs were allowed to dry. At chosen intervals 5 moist and 5 dry discs were selected at random from chambers exposed at 30°, 33°, 36°, and 39° C. The discs were planted on nutrient agar immediately following their removal from treatment chambers and growth of the fungus was later recorded.

For a further test of the survivability of the fungus, branches 3 to 5 inches in diameter were obtained from a naturally inoculated diseased elm

and these were cut into a number of 3-inch-long sections without removing the bark. The exposed wood surfaces of about one-half the number of sections were covered with paraffin to prevent excessive changes in water content; those of the remaining sections were left unprotected. Seven lots were prepared and each was stored as shown in table 7. At intervals of two months a sample was taken from each lot. Tissue with fungus discoloration was dissected from each sample; some of it was planted on acidified and some on nonacidified potato-dextrose agar and growth was later recorded.

RESULTS

The data presented are representative results obtained by repeating each of the different experiments several times except for those obtained from the 2-year survivability test which was not repeated.

Effect of Temperature

Spore germination. The coremiospores of Ceratostomella ulmi germinated best at 27° C.; at this temperature the percentage of spores germinated

TABLE 1.—Effect of temperature on the germination of coremiospores of Ceratostomella ulmi

					P	er cent ge	rminate	da			
Race	Committee of the second					Tempera	ure, ° C.				
	3	6	9	12	15	18	21	24	27	30	33
B	0	0	0	0 Tr	Tr ^b Tr	Tr Tr	75.6 73.6	93.4 96.1	97.0 98.6	94.0 95.6	$\frac{\mathrm{Tr}}{\mathrm{Tr}}$

^{*} After an incubation of 40 hours the cultures held at 18°, 15°, 12°, 9°, 6° and 3° C. were budding; budding was profuse at 18° and tapered off to a bare trace at 3° C.

b Tr = trace.

for each of two representative cultural races was respectively, 97.0 and 98.6. In general, spores germinated well from 21° to 30° C., inclusive, during 13 hours. Very few spores germinated at 12°, 15°, 18°, and 33° C. At 18° C. and lower temperatures the spores tended to bud in a yeast-like manner; budding was profuse at 18° C. and scanty at 3° C. (Table 1).

The organism was rather capricious in regard to spore germination and at least three factors appeared partly responsible. First, probably because of inherent differences the spores of some races germinated in greater numbers than did those of others. Second, spores that were taken from 10- to 15-day-old coremia usually germinated better than those from younger or older fruiting structures. Third, the nutrient medium was important because spores germinated much better in a solution of technical grade maltose than they did in a solution of chemically pure maltose. Since pyridoxine (3, 7, 8) and certain minor elements² appear to be essential to the growth

² Pope, Seth A. Unpublished data. Plant Pathology. Cornell University. Ithaca, New York.

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of *Ceratostomella ulmi* it is possible that these or other similar growth-promoting substances existed in the technical grade maltose but were absent from the chemically pure sugar.

Growth of mycelium. Vegetative growth of the fungus on potato-dextrose agar was more rapid at 27° C. than at any of the other temperatures (Figs. 1 and 2). The temperature range favoring generally rapid growth on solid media extended from 21° to 30° C., inclusive, and it is recalled that these limits marked the range most favorable for spore germination. Growth occurred at about 3° and 33° C., but not at 1° C. nor at 36° C. Decrease

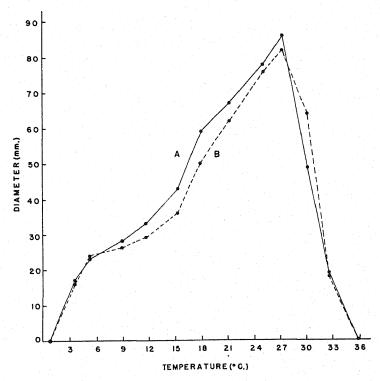


Fig. 1. The effect of temperature on the growth of Ceratostomella ulmi, cultural races A and B, on potato-dextrose agar.

of the growth rate at temperatures above 30° C. was extremely abrupt while the depression was gradual from the optimum of 27° C. down to about 3° C. (Fig. 1).

Colonies of the fungus grown at 12° C. or lower, and at 30° C. were distinctly white in contrast to the pale greyish cream color of those grown at more favorable temperatures. Conidiophore development of the "Cephalosporium type" was pronounced at favorable temperatures but colonies grown at temperatures less favorable were mostly without aerial conidiophores. The latter type of colony was composed of compact, irregular hyphae and many yeast-like cells. The fungus races used responded simi-

larly to temperature and each maintained its distinctive cultural characters at temperatures in the range that favored growth.

Growth of coremia. The optimum temperature for development of coremia on elm-wood discs mounted on non-nutrient agar was about 24° C. (Table 2). In cultures examined after they had grown for 4 days coremia had developed in those of race C, from 21° to 30° C., but not in those of other races. In thirty days coremia formed in cultures of race C at all temperatures from 3° to 30° C., but not at 33° C.; there were very few formed during this period at 6° C. and lower. Races F and D failed to produce coremia at any temperature tried and this result conforms with all past observed behavior of these races in relation to the production of coremia.

TABLE 2.—Effect of temperature on coremial development by three different races of Ceratostomella ulmi. Diseased wood mounted on non-nutrient agar

				R	elativ	abu	ndan	cea of core	mia after	
Temp.,				4 days	3				30 days	
°C.				Race	*************************************				Race	
		F		Q		D		Fр	C	Dq
3		***	-					-	-+c	 _
9 12						=		_	+ +c + + +	_
15 18				_				_	+++	_
21 24		_ '		+ ++		_		= = = = = = = = = = = = = = = = = = = =	+++	_
27 30 33		_		-+		- -			+++ +++	· ·

^{*} Number of coremia per wood section: -= none; -+-=1 to 5; -+=6 to 10; += 11 to 20; ++= 21 to 30; +++= more than 30.

b Mycelium present at all temperatures but very scanty at 3°, 6°, 9° and 33° C.

Results essentially similar were obtained when discs of diseased elm wood were placed on moist cotton wicks (Table 3). In addition to race C, used in the first experiment, two other coremium-forming races, B and E, were used. Temperatures of 6°, 3°, and 1° C. appeared to inhibit the formation of coremia by the two latter races and in this respect they differed from race C which had the capacity to form coremia even at 3° C.

The temperature optimum for coremium development did not appear to be the same as that inducing the highest percentage spore germination and the most rapid mycelial growth (cf. Table 1, Fig. 1, and Table 2). Visual evidence of this for race B is in figure 2; the dark concentric rings in cultures held at 21° and at 24° C. were due to the presence of coremia. On potato-dextrose agar the coremia formed best at 24° C., fairly well at 21° C., but they formed sparingly or not at all at the other temperatures during the 8-day exposure.

d Mycelium present at all temperatures except 33° C. and very scanty at 3°, 6°, 9° and 12° C.

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TABLE 3.—Effect of temperature on coremial development by three different races of Ceratostomella ulmi. Diseased wood mounted on cotton wicks

		Relat	ive abunda	ncea of coremia	after	
Temp.,		4 days			20 days	
°C. ′		Race			Race	
·	В	С	E	В	C	Е
1		-		-	-	
6		<u> </u>			· · · · · 🗆 🗆 · · · · ·	
$^9_{12}$	_	. i		- - -		-
$\frac{12}{15}$			_	-+-	-+-	+
18		_	· ·	-+	+	++
$\frac{21}{24}$	+		-+	++	+ ++	++
27	-+-	-+-	-	++	++	+++
30 33	+			+++	++	+++

* Number of coremia per wood section: -= none; -+-=1 to 5; -+=6 to 10; +=11 to 20; ++=21 to 30; +++= more than 30.

After 80 days the lowest temperature at which coremia were formed by each of the different races was: B, 12° C.; C, 3° C.; E, 9° C. None of the races formed coremia at 33° C. but there was very scanty growth of mycelium.

Effect of Humidity

Spore germination. Coremiospores of Ceratostomella ulmi germinated within 24 hours on glass slides at relative humidities of approximately 98 and 100 per cent (Table 4). Those exposed at approximately 95, 93, 90, and 85 per cent relative humidity failed to germinate. At the end of 48 hours spores that germinated at 98 and 100 per cent relative humidity respectively had formed long, vigorously branching hyphae.

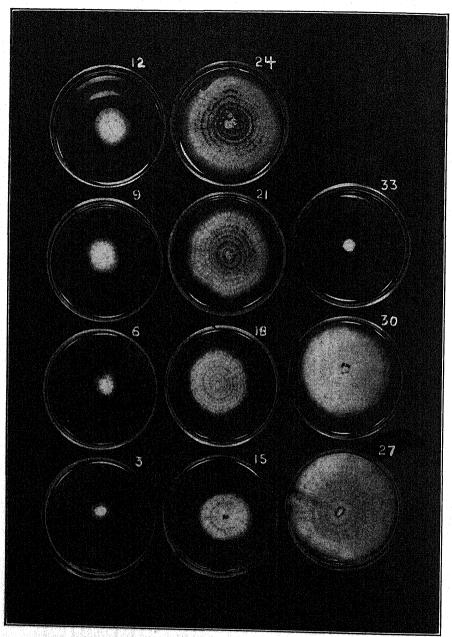
Mycelial growth and fruiting. The growth of Ceratostomella ulmi in and on elm wood was markedly influenced by air moisture (Table 5). In

TABLE 4.—Effect of humidity on germination of coremiospores of Ceratostomella ulmi

Lower Hours' exposure	Slide surface Upper		
Hours' exposure	TT annual arrangement	10	
Trours exposure	Hours' exposure	,	
24 48	24 48		
+ ++			
+ TT	T		
		+ ++ ++ + ++ ++ 	

^{*++=} excellent germination with long branching hyphae and numerous conidia; += good germination with less branching and fewer conidia; -= no germination.

b Negative results were obtained also at 85 and 93 per cent relative humidity.



#16.2. The effect of temperature (°C.) on the growth of Ceratostomella ulmi, race B, on potato-dextrose agar.

general, growth of the fungus was good in an atmosphere whose relative humidity was about 98 per cent or higher, both on healthy elm wood planted with spores and in invaded or diseased wood. At 96 per cent relative humidity and lower the organism failed to develop on healthy elm wood planted with spores, but it did grow out from diseased wood. Coremia developed on diseased wood at 96 per cent relative humidity and higher but on elm wood planted with spores they did not develop at a relative humidity lower than 98 per cent. From these observations it appears that if the organism exists as hyphae in the wood it is far more likely to grow when moisture of the surrounding air is lowered than it is if in the form of scattered spores on the outer surfaces of wood.

TABLE 5.—Effect of air humidity on the growth of Ceratostomella ulmi on elm wood. Exposed at room temperature.

		Type ar	nd relative a	mount of growt	h ^a			
Per cent relative	Pla	nted elm wo	od	Diseased elm wood				
humidity	Coremia	Conidia	Mycelia	Coremia	Conidia	Mycelia		
92 96	_	_	_		-+	-+		
98 100	-+ +b	+++	+	+ +c + +c	+++	++		

Effect of Temperature and Moisture on Survival

Temperature and moisture exerted a pronounced influence on the survivability of Ceratostomella ulmi. In small discs of diseased wood that were allowed to dry the fungus survived 8 and 6 days at 30° and 33° C., respectively, while in wood kept moist it was still alive at the end of 14 days (Table 6). In moist wood at these temperatures it is likely that the organism would live much longer. At 36° and at 39° C., C. ulmi lived in wood kept moist for 8 and 4 days respectively, while in wood allowed to dry the survival periods were 4 and 3 days respectively. Thus moisture aided C. ulmi to survive in wood at higher temperatures, but this protective effect rapidly diminished with increase in temperature above 33° C.

In another experiment coremiospores were suspended in sterile, distilled water and sprayed on hot-air-sterilized elm wood. The planted wood was placed in test tubes plugged with nonabsorbent cotton and capped with cellophane to prevent excessive accumulation of moisture in and on the wood. Some of the cultures were stored constantly at -10° C.; others were held constantly in a room whose temperature fluctuated between 12° and 20° C. At chosen intervals wood bearing spores was removed from the tubes, the

b Small coremial heads.

c Large coremial heads.

TABLE 6.—Effect of temperature and moisture on the survival of Ceratostomella ulmi in small discs of diseased wood

					Day	sur	viving	in dis	eased	wooda				
Temp.,				Moist							Dry			
	2	3	4	6	8	10	14	2	3	4	6	8	10	14
30 33 36 39	+++++	+ + + + + + + + + + + + + + + + + + + +	+ + -+	+ + -+	+ -+ -+	+ +	+ + -	+ + + +	+ -+ -+ -+	-+ -+ -+	-+ -+	-+	- - - - - - -	

⁺⁼ organism survived in all samples from given lot.

-= no survival; indicates no test.

spores were washed from the wood and suspended in maltose (1.5 per cent) solution that was acidified to prevent the growth of bacteria. Drops of the spore suspension were placed on glass slides mounted in moist chambers that were kept for 12 to 14 hours at 25° C. The viability of spores from each of the treatments rapidly declined. At the end of one month about 8 per cent of the spores from each treatment were germinable. After $4\frac{1}{2}$ months about 2 per cent of the spores exposed at -10° C. were still viable, while those exposed at 12° to 20° C. failed to germinate at the end of $1\frac{1}{2}$ months. About 5 per cent of the spores taken from coremia that were allowed to dry at the same temperatures retained their germinability for $4\frac{1}{2}$ to 5 months.

In larger sections of wood cut from naturally diseased elm, *Ceratosto-mella ulmi* survived as long as two years (for the duration of the test) under conditions that prevented rapid desiccation (Table 7). On the other hand, the fungus succumbed in about 6 months in wood that was allowed to dry rapidly in a shed that protected it from rain. Moderate freezing tempera-

TABLE 7.—The effect of storage conditions on the survival of Ceratostomella ulmi in diseased native elm wood

Storage treatment of wood sections	Survival period in months ²
Nonparaffined Stored about 6 feet above the floor of a rain-proof tool shed in an open container	6
Stored in an open container and exposed alternately for one week at a time to -10° C. and then 15° C. Stored at -3° C. in an open container	20 24
Paraffined Stored outdoors about 6 feet above ground level in an open porous container	20
Stored at -10° C. in an open container	22
Buried in the saud floor of an outdoor screen-covered cage to a depth of 1 the diameter of the sections	24
Stored in an open container and exposed alternately for one week at a time to -10° C, and then 15° C,	24

^{*} The test continued for 24 months.

⁻⁺⁼ organism survived in part of samples from given lot.

tures such as those that prevail at times during the winter in the New York City area did not appear to significantly shorten the survival period of the fungus. In fact, constant storage of diseased wood at temperatures of -3° , and -10° C. apparently helped to conserve the moisture in the wood necessary for the fungus.

Alternate freezing and thawing alone did not appear to have a pronounced deleterious effect on the organism. Under such conditions the organism lived longer in paraffined wood than it did in nonparaffined wood presumably because moisture was conserved in the former.

Competing organisms did not appear to materially affect the survival of *Ceratostomella ulmi*. In fact, it survived the 2-year period in wood that was partially buried in sand outdoors and this treatment was much more conducive to the development of competitive saprogens than any of the other treatments.

DISCUSSION

Ceratostomella ulmi grows best at moderately high temperatures. Despite this fact its growth activities are markedly slowed by temperatures above 30° C. and they practically cease at 33° C. or slightly above. While growth is possible over a rather wide range of temperature the organism is best adapted to that from 18° to 30° C. and this adaptation seems admirably suited to the saprogenic growth of the fungus in the areas of the United States where it prevails as a pathogen.

At temperatures below the range most favorable for mycelial growth, the organism can increase by budding. This phenomenon might help to intensify the Dutch elm disease in at least three ways: through an increase in the amount of inoculum at the source; through an increase of inoculum at the infection court; and through increased numbers of spores within the xylem vessels where they move freely to bring about rapid invasion of the tree (1, 5).

Moisture is a primary factor that may limit the saprogenic activities of Ceratostomella ulmi. Low air and substrate moisture may not only inhibit germination of spores but it may also suppress the growth of hyphae and the production of fruiting structures. Since the organism depends largely, for its continued existence and distribution, on the inoculum developed in pupal cells made by its principal insect vectors the suppression of this growth activity may well be critical. The degree to which the moisture factor is felt by the organism depends greatly upon its location and state of association with elm material. For example, in the form of spores and/or hyphae on the outer surfaces of elm wood the organism is unable to grow if the relative humidity of the surrounding air is less than 98 per cent. However, it can grow out from wood in which it became established in the form of hyphae during the disease process even if such material is kept in an atmosphere whose relative humidity is as low as 92 per cent. Thus, it appears that the latter more vital type of association enables the organism to obtain some water from the surrounding wood even when the diseased wood is exposed in relatively dry air.

Once the Dutch elm disease fungus becomes established in elm wood it is capable of persisting as a saprogen for rather extended periods under a variety of conditions. From the disease control viewpoint this is unfortunate because it means that dead, dying, and decadent elm material of all sorts that has not been debarked may constitute a source of inoculum. However, the inability of the organism to withstand rapid and prolonged drying, even when embedded in wood, is a characteristic that renders it vulnerable. Perhaps more concerted effort to make use of this fact in connection with sanitation work would be a forward step toward more satisfactory control of the Dutch elm disease.

SUMMARY

The optimum temperature for the germination of coremiospores of 2 cultural races of Ceratostomella ulmi was about 27° C. At 18° C. and lower the spores tended to bud. Other factors such as racial differences, age of spores, and nutrition affected spore germination.

The temperature range generally favorable for growth of the mycelium on potato-dextrose agar was 21° to 30° C. For the cultural races studied, the minimum temperature for growth lay between 1° and about 3° C., the optimum temperature was about 27° C., and the maximum was between 33° and 36° C.

The optimum temperature for development of coremia was about 24° C. Some cultural races formed coremia on elm wood at temperatures that ranged from 3° to 30° C., others did not form them below 9° C., while two races did not produce coremia at any temperature tried.

Coremiospores of Ceratostomella ulmi germinated well on glass microslides and on elm wood at relative humidities of 98 per cent and higher; they did not germinate at 96 per cent and lower.

Vitally established in the form of mycelium and spores in diseased elm wood the organism grew out over the surface at relative air humidities of 92 per cent and higher. Growth was very sparse at 92 per cent, fair at 96 per cent, and abundant at 98 and 100 per cent relative humidity.

Ceratostomella ulmi lived at least 2 years in diseased elm wood that was protected from rapid loss of water and from abnormally high temperatures. Conditions that induced and permitted rapid loss of water from diseased wood were generally unfavorable for the survival of the pathogen in such material.

DEPARTMENT OF PLANT PATHOLOGY. CORNELL UNIVERSITY. ITHACA, NEW YORK.

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ANTHRACNOSE RESISTANCE IN FLAX¹

CHARLES RAY, JR.2

(Accepted for publication April 10, 1945)

Anthraceose of flax, caused by Colletotrichum lini (1, 3, 4, 5, 6, 7), is distributed throughout the flax-growing regions of the world. The disease first caused an appreciable loss in California flax in the season of 1940–1941 (5, 6). Houston (6) has estimated losses as high as 35 per cent in some fields during that season. Although recent losses have not been as marked, the organism is still present and could, when the season is favorable, again become destructive, especially since the flax variety still predominant in California, Punjab, is very susceptible to attack. In the North Central States, although flax anthraceose has acted as a seedling blight and canker and has considerably damaged developing plants in local areas in Minnesota, it has not been regarded as being especially serious (3).

Little has been published concerning the reaction of current flax varieties to Colletotrichum. The susceptibility of Punjab and the partial resistance of Argentine (C.I. 463) have been known for several years. More recently Houston reported (6) that Rio (C.I. 280) and the plant introductions C.I. 1008 and C.I. 1009 also have partial resistance. He reported that C.I. 1008 and C.I. 1009 carry a very high degree of resistance.

This paper records the anthracnose reactions of a number of flax varieties.

MATERIALS AND METHODS

The inoculations were on plants grown in 5-inch pots in the greenhouse from November, 1943, to March, 1944. Supplementary tests have been made from November, 1944, to February, 1945. The seed were planted in steam-sterilized Toxaway silt loam. The seedlings were thinned to 15 per pot. Duplicate pots were tested when the seedlings were 5-8 inches tall.

The culture of *Colletotrichum lini* was furnished by Dr. B. R. Houston,³ to whom we extend our thanks. The culture has been increased on potatodextrose agar.

Vigorously growing cultures of the fungus, about 10 days old, were used as a source of spores; and enough spores were scraped from the agar slants into distilled water to make the water turbid. The stems and leaves of the seedlings were sprayed (4, 7) by means of a small atomizer. A small air pump connected to the atomizer aids in securing a heavy, uniform inoculation. The susceptible Punjab variety of flax was included in each group of inoculated plants as a check upon the effectiveness of inoculum. After inoculation, the seedlings were immediately placed in a moist chamber and

¹ Contribution from the Plant Research Department, California Central Fibre Corp., Pisgah Forest, N. C.

² Geneticist.

³ Junior Plant Pathologist in the Experiment Station, University of California, College of Agriculture.

left for about 48 hours (Fig. 1) after which they were returned to the bench in a moist greenhouse. Observations were made 12–15 days after spraying at which time the symptoms were usually well developed.

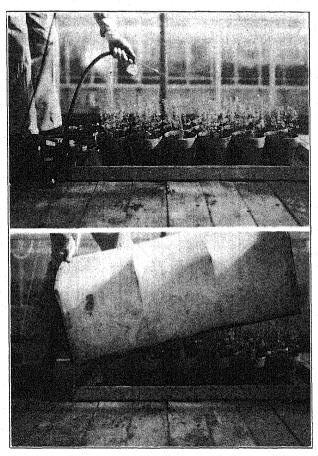


FIG. 1. Flax seedlings 5-8 inches tall are sprayed with a water suspension of spores of *Colletotrichum lini*. A small air pump aids in securing a heavy, uniform inoculation. After spraying, the inoculated seedlings are kept for about 48 hours under a cloth-covered frame which is kept moist.

RESULTS

The anthracnose on inoculated varieties ranged from severe infection of hypocotyls, stems, and leaves with much plant killing to very few small lesions on but few of the leaves (Fig. 2). No variety yet examined is regarded as being completely immune. For convenience, the varieties were placed in one of four groups according to the degree of infection: Group I—those with few, small leaf lesions but with no stem lesions and no plant killing (Fig. 2, D); Group II—those with a moderate number of somewhat larger leaf lesions and with some but not extreme leaf killing and with no stem lesions (Fig. 2, C); Group III—severe leaf infection with many leaves

attacked and large, rapidly spreading lesions causing much leaf killing. Stem lesions were present in varying amount (Fig. 2, B). Group IV—those with severe leaf and stem lesions. The stem lesions usually girdled the stem and killed the plant (Fig. 2, A).

Table 1 summarizes the results of the inoculations with *Colletotrichum*. The samples tested have been assigned to one of the four groups.

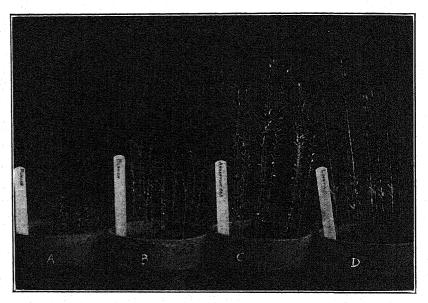


Fig. 2. Punjab, Blenda, Argentine 463, and Linota flax 14 days after inoculation with Colletotrichum lini. A. Very susceptible (Group IV). B. Susceptible (Group III). C. Moderately resistant (Group II). D. Resistant (Group I).

DISCUSSION

Table 1 records the amount of injury observed after spraying seedlings with a water suspension of spores of *Colletotrichum lini* and demonstrates that in these seedling tests the varieties differed in the amount of disease appearing. The results have been used to classify the varieties tentatively as resistant or susceptible as follows: Group I—resistant; Group II—moderately resistant; Group III—susceptible; Group IV—very susceptible.

It is believed that this classification according to resistance should not be too rigidly applied, especially for the intermediate classes. There are several reasons for caution. Primarily, our information on environmental effects is meager. Little precise information is available concerning the effect of soil or air temperature, moisture, light, or soil amendments on the virulence of the disease. Further, it is not recorded to what extent physiological races of the organism exist. The classification of some varieties is difficult, especially when considering the amount of leaf spotting. Different varieties grade from very susceptible to very resistant.

Greenhouse disease studies usually provoke the question of the applica-

TABLE 1.—Amount of anthracnose injury 12-15 days after spraying flax seedlings with a water suspension of spores of Colletotrichum lini

Variety	C.I.ª No.	P.R.b No.	Class reaction
Argentine	472	71–1	I
do	472	71-2	1
do	462	72–2	I
Buda	326	131-1	Ī
Buda 80	873	132-1	Î
	982	22-1	İ
Crystal			
do	982	22–2	Ī
Golden × Rio	980	30–3	Ι
Linota	244	82-1	I
Malabrigo	696	104-1	1
do	696	104-3	I
N. D. 40046	492	84–1	ī
Portuguese Sel.	1021	198	Ī
Rio	280	14-1	Ĩ
unnamed	1008	718–1	Ι
do	1009	719–1	I
do	1009	719-2	I
Bison	389	3-1	I-IId
		• •	
Argentine	463	73-2	II
do	463	73-4	ĨĨ
do	463	73–22	II
Goar Sel. No. 5	1112	750-1	II
Cascade		794	II
Blenda Line		367-21	II-IIId
Common Pink	479	777	II-IIId
Fleischman		445-21	II-IIIa
Giza	378	83-2	II-IIId
G12a	910	00-L	11-111-
Abyssinian	36	68-21	III
do	300	69	ĨĨĨ
Ac. 179		793	iii

Blenda Line		367-1	III
do	.,	367-2	III
do	***********	367-3	III
B. Golden Sel.	976	33-2	III
do	976	33-4	III
do	977	34-1	III
do	977	34-2	ÎÏÎ
<u> </u>			
Cirrus	727	411-1	III
Hercules	***************************************	332–4	111
do	************	332-21	III
J. W. S.	800	146– 1	III
J. W. S. × Bison	1043	29-1	III
Liral 12	******	487-1	III
Martin	1062	333-31	III
		124-1	ÎÎÎ
Morocco	376		
Newland	188	138-1	III
Pinnacle	693	190-2	III
do	693	190-4	III
Redwing	320	12-1	III
Royal	828	16	III
Russian fiber		478-4	III
	687-1	41-1	ΪΪ
Talmune			
do	687-1	41-21	ĨIĨ
Texala	***************************************	485-4	III
Victory	1045	2-1	III
đo	1045	2-2	III
do	1045	$\bar{2} - \bar{3}$	ΪΪΪ
termina a	981	10	ΪΪΪ
		796	III
No. 98701 No. 99407	***********	795	III

TABLE 1.—(Continued)

Variety	C.I.ª No.	P.R.b No.	Class reactione
Black Mountain		88	III-IVa
B. Golden Sel.		33-1	III–IVd
Stamm 8		355-1	III-IVa
Abyssinian		68-1	IV
do	0.00	70-1	IV
Albidum Type 12		59	IV
Bold		54	IV
Bridgemohan Ganj		66	IV
Buxar		64	IV
Concurrent		330-2	IV
do		330-21	IV
Dutch Hybrid		376-1	IV
Giza		83-11	IV
Gossamer		371 - 4	IV
Hoshangabad	. 40	126	IV
Indian Type 2		61	IV
do 4		60	IV
do Sel. 6do 121		58	IV
do 121		143	IV
Ismailpur		57	IV
Pinnacle		190-22	ĪV
Punjab	. 20	48-1	IV
ďo	20.44	49-2	IV
do	. 20	49-7	ĪV
Rafigang		56	īv
Sitamarhi		67	ĪV
Stamm 8		322-1	īv
Talmune		41-2	ĩv

^a C.I. refers to accession number of the Division of Cereal Crops and Diseases, U. S. Dept. Agr.

b P.R. refers to accession number of the Plant Research Department, California Central Fibre Corp. Numbers following dash indicate selected strains.

Refers to Group number described in text and illustrated in figure 2.

d Somewhat intermediate between the two groups.

bility of the indoor observation to field conditions. There are, however, numerous instances in small grain work where the agreement between field and greenhouse disease observation is close. There are only a few recorded observations of the field reaction of flax to Colletotrichum lini which can be compared to greenhouse studies. The high susceptibility of Punjab flax to Colletotrichum in the field in California is well known and conforms to our greenhouse observation. A number of Indian types of flax were badly injured by Colletotrichum in our experimental field plots in 1942; these types were very susceptible in our greenhouse tests. Flor (2) records some observations on Colletotrichum in the field during 1944. He recorded the presence of leaf lesions, apparently caused by Colletotrichum lini, on Crystal flax near Morris, Minn., but stated that the anthracuose was not in epidemic form, and that it was not clear whether the poor stands were due to frost, weak germination, or seedling blight. Our greenhouse tests showed Crystal to have but few small leaf lesions after inoculation with Colletotrichum. Flor also records somewhat more abundant lesions of C. lini on Royal flax at Morris, Minn., and in a field of Victory near Grafton, North Dakota. Our greenhouse results rate both Royal flax and Victory flax as somewhat more susceptible than Crystal. The greenhouse reaction of flax to *C. lini* is thus not inconsistent with the few field observations at hand. More data, however, are desired to throw more light on this question of field *vs.* greenhouse observations.

Nevertheless, the observations recorded in table 1 demonstrate that there are marked genetic differences between flax varieties in the amount of resistance to *Colletotrichum lini*. During the tests, Punjab flax seedlings were nearly always included as a check upon the severity of the inoculation. Under the conditions of these tests, the inoculation was always heavy enough to kill the Punjab seedlings.

Several generalizations are drawn from the data presented. None of the Indian varieties tested—Albidum, Bold, Bridgemohan Ganj, Buxar, Hoshangabad, Indian Types 2, 4, 6, 121, Ismailpur, Punjab, Rafigang, and Sitimarhi—have had any resistance to Colletotrichum. Various samples of Punjab have been tested repeatedly as checks, yet none has had any resistant plants. This would indicate that so far as anthracnose resistance is concerned no profit can be derived from individual plant selections of Punjab and, if a Punjab type is best in the California area, then the most likely approach would be through use of hybridization.

The Argentine group of flax varieties current among breeders in the United States carry some resistance to anthraconse.

As a group, the fiber flax varieties included in our tests seem to have little anthracnose resistance, though a few were classified in the moderately resistant Group II. None were classified as Group I. This result is contrary to the report of Hiura (4) who came to the conclusion that the broad-leaved varieties of seed flax are very susceptible to anthracnose, while the narrow-leaved varieties of fiber flax are comparatively resistant.

The existence of varieties carrying resistance to anthracnose—even under our extreme tests-encourages the conclusion that improved varieties can be obtained by hybridization. The large-seeded Argentine types, the taller seed varieties such as Linota, Bison, and Buda, the unnamed introductions C.I. 1008 and C.I. 1009, and others furnish a range of types of initial breeding material. Our results in breeding, to be published in a later paper, indicate that resistance is not too difficult to transfer. The results in general confirm the grouping in table 1. Crosses of the susceptible Punjab with the more resistant varieties, Argentine 462, Argentine 463, Argentine 472, Rio, Buda 80, Bison, Malabrigo, Linota, C.I. 1008, and C.I. 1009, segregated for resistance and susceptibility in the F2; resistant and susceptible lines have been found in the F₃. Crosses of the moderately resistant Argentine 463 with susceptible Gossamer, Pinnacle, Martin, Talmune, and Cirrus also segregated in the F₂. Crosses of Punjab with susceptible Dutch Hybrid, Pinnacle, Cirrus, and Abyssinian gave a completely susceptible F₂. Crosses of resistant Argentine with resistant C.I. 1008 and C.I. 1009 did not throw any susceptible plants in the F₂. C.I. 1008, C.I. 1009, Linota, and Buda 80 appeared especially good in the variety tests for anthracnose resistance and have given good segregates in hybrids.

SUMMARY

The method used for testing seedlings of flax with Colletotrichum lini is described and the varieties are classified according to the amount of injury following inoculation. A range of types carrying anthracnose resistance is available for breeding material. C.I. 1008, C.I. 1009, Linota, and Buda 80 were especially free from anthracnose. Punjab in repeated tests has never had any resistant plants. Argentine types carry various degrees of resistance.

PLANT RESEARCH DEPARTMENT, CALIFORNIA CENTRAL FIBRE CORPORATION, PISGAH FOREST, NORTH CAROLINA.

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A GRAFT-TRANSMISSIBLE VIRUS OF SWEET POTATO

S. P. DOOLITTLE 1 AND L. L. HARTER 2

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While examining sweet-potato plots at Beltsville, Md., during the summer of 1942, a plant was found with symptoms indicating a virus infection. This plant was one of a variety designated as No. 029878 that had been propagated from roots received from Leningrad, U.S.S.R., in 1934. No other plants of this variety showed any evidence of the disease in 1942 and none had been noted in previous years. An examination of the remainder of the planting failed to show any evidence of similar infection in the many other varieties included in the trials. Cuttings were made from the supposedly mosaic plant and were rooted in the greenhouse, where a stock has since been vegetatively propagated for observation and experimental use.

SYMPTOMS

The symptoms on the original plant were typical of those associated with viruses of the mosaic (Marmor) group. These same symptoms have appeared consistently in artificially infected plants during two years' observations in the greenhouse. In many plants the primary symptom is a pronounced yellowing along the veins of the younger leaves. This yellowing may outline many of the very small veins in a manner suggestive of frost patterns on a window pane (Fig. 1, A), or may affect only certain sections of the veins as shown in figure 1, B. On other plants, the first symptom may consist only of small, diffuse, yellow spots from 1 to 2 mm. in diameter (Fig. 1, C). These spots, however, frequently occur together with some vein-clearing of the type mentioned. Symptoms of both types also are found on leaves produced after the plant has been infected for some time.

In later stages of the disease, the yellowing of the veins is often followed by the development of pale green areas that may occur as comparatively small spots or may be so large as to include one-third to one-half of the leaf (Fig. 1, D). In such leaves the darker portions often are abnormally dark green and may show small, feathery areas of yellow along the veins. The leaves are sometimes slightly rugose and dwarfed but not much distorted. Infected plants commonly have shortened internodes, and there is a definite stunting of the plant in late stages of the disease (Fig. 2). No necrotic symptoms have ever been noted on leaves, stems, or roots. The fleshy roots are smaller than those of healthy plants but infected plants in the greenhouse always produce a fair number of medium-size storage roots.

When mosaic plants grow slowly, the leaf symptoms are likely to be less pronounced than in rapidly growing plants. When growth is slow, there

² Formerly Senior Pathologist in the same division.

¹ Senior Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

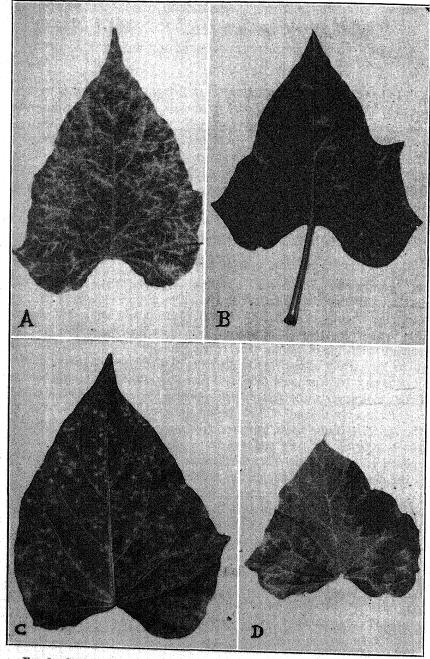


Fig. 1. Sweet-potato leaves with symptoms of mosaic. A, B, C.—Symptoms occurring in early stages of the disease and appearing to some extent on later growth. D.—Leaf with large light and dark green areas produced some time after infection.

usually are some leaves with only faint evidence of the disease but there is no general masking or extreme mildness of symptoms in the leaves of such plants. When plants are grown in the greenhouse at summer temperatures ranging from 75° to 105° F., the mottling is fairly pronounced. Temperatures as low as 60° to 65° F. do not suppress mosaic symptoms in the new growth.

The occurrence of mosaic symptoms on sweet-potato plants has been reported by various observers, chiefly in the Southern States. In many of these reports the symptoms were not described and, in all instances where descriptions are given, the symptoms of the disease appear to have been quite different from those produced by the virus with which we have been working.

Rosen (5, 6) reported a mosaic disease of sweet potato in Arkansas that appeared to be confined to the variety Nancy Hall. The leaves of diseased



Fig. 2. Nancy Hall sweet-potato plant (at right) showing stunting characteristic of late stages of mosaic infection. Inoculated by root-plug method. Healthy control (at left) grown from uninoculated half of same root.

plants commonly showed a marked narrowing of the blades and an irregularity of the margins, accompanied by a pronounced puckering of the leaf. The affected leaves also showed a mottling consisting either of very small, light-colored dots, streaks, and blotches generally distributed over the leaf, or of larger, irregular blotches of light green occurring between areas of normal color. The leaf symptoms of these plants, as shown in the colored plate and text figures of the two papers, are unlike the symptoms of the disease we have studied at Beltsville. Rosen reported (6) that the virus found in Arkansas was transmissible by rubbing the leaves with extracted juice of mosaic plants and by injecting the mosaic juices into the stem. No evidence of infection was obtained, however, except in a few instances where the inoculated plants were kept in continuous growth for 12 to 13 months. The plants were inoculated in the field during the spring and carried over winter in the greenhouse by means of cuttings that were again set in the field during the following spring. When the fleshy roots of inoculated plants were used for propagation, after storage during the winter, no evidence of the disease occurred during as many as three successive seasons.

grafts and contact of cut surfaces of diseased and healthy roots did not produce infection in healthy plants.

Weber (7) described a mosaic disease, in Florida, that caused a stunting and rosette-like appearance of the affected plants. The larger veins of the leaves were wide and flattened in a way suggestive of fasciation. blades were narrow and filiform, with pouch-like protuberances between the There was little evident mottling veins and a crinkling of the leaf margins. of the leaves, but in other respects the symptoms appear to have been much like those described by Rosen although of a more severe type. No attempts were made to infect healthy plants by grafting but mechanical inoculations with the expressed juice of diseased vines were not successful. paper (8) Weber and West reported what they considered to be a second type of mosaic on sweet potato but made no mention of any attempts to transmit a virus from diseased to healthy plants. The symptoms of this second disease included a yellowing of the veins which, in some respects, was not unlike that caused by the virus we have studied. However, there was a wrinkling and pouchiness of the leaf blades that we have never observed and the later mottling of the leaves appears to have been of a somewhat different type from that occurring in the infected plants of our experiments.

Weimer (9) studied a mosaic-like disease on the variety Nancy Hall but was unable to secure infection by mechanical inoculation with expressed juices of diseased plants. Grafting of diseased and healthy stems and the grafting of healthy shoots on diseased roots also failed to cause infection. He did not describe the symptoms of the diseased plants used as a source of inoculation but it is known that they were much like those reported by Rosen.

Harter and Whitney (3), working with the variety Nancy Hall, found that an air temperature of 38° C., either with or without high humidity, would cause complete masking of symptoms in plants affected with a disease much like that described by Rosen and apparently identical with the one studied by Weimer.

Wellman (10) reported that he was able to transmit the southern celery strain of ordinary cucumber mosaic to sweet potato by means of aphids and could recover the virus by the same means. Mechanical inoculations gave only negative results. The first symptoms consisted of a vein-clearing that appeared about 7 days after inoculation. This clearing of the veins later disappeared and was followed by a mild but definite mottling that became almost undetectable in plants approaching maturity. He stated that this disease was found on sweet potatoes in the field in Florida and Cuba. Wellman showed no photographs of symptoms but the disappearance of the vein-clearing and the eventual fading of the mottling of the foliage are unlike the symptoms of the disease that we have studied. The incubation period of the celery virus on sweet potato was also much shorter than any observed in our inoculation experiments.

In a recent report, Hansford (2) states that surveys made in 1944 show that sweet potatoes in certain sections of East Africa frequently are affected with what appears to be a virus disease. The symptoms, which vary with the variety affected, include mottling, vein-banding, distortion or crinkling of the leaves, and some stunting of the plants, especially those of narrow-leaved varieties. Preliminary surveys indicate that the disease is of considerable importance. Nothing is yet known as to methods of transmitting the suspected virus but the spread of the disease in the field indicates the presence of an insect vector. The symptoms described by Hansford are suggestive of those of the disease found at Beltsville but no definite comparison can be made at this time.

TRANSMISSION OF THE VIRUS

Inoculations with Juices of Mosaic Plants

All attempts to secure infection by inoculation with expressed juices or crushed tissues of mosaic plants have been unsuccessful. A total of 103 plants were inoculated by rubbing the leaves with the expressed juices of mosaic leaves. Leaves of various ages were used as a source of inoculum and all inoculated leaves were dusted with carborundum powder before rubbing. Similar leaf inoculations were also made with the juices of stems, and of the feeding and the storage roots of mosaic plants.

Stem inoculations were made (a) by introducing expressed juices of mosaic stems or leaves into the stem by needle pricks or by injection with a hypodermic needle, (b) by the insertion of freshly macerated tissue of stems or leaves into longitudinal incisions in the stem, and (c) by the hypodermic injection of juices extruded from the ends of freshly cut stems of mosaic plants. A total of 92 plants were inoculated by one or another of these methods but none developed symptoms of mosaic.

All of the plants inoculated were held for periods of from 6 weeks to 8 months at greenhouse temperatures between 75° and 95° F. Since plants infected by grafting show symptoms within 14 to 28 days after the graft is made, it appears that infection is not readily obtainable by inoculations with expressed juices.

Transmission by Stem Grafting

Attempts at transmission of the virus by approach-grafting of stems of mosaic and healthy plants have been uniformly successful where union of the stems has occurred. The healthy and mosaic stems were joined by tongue grafts made about 4 to 6 inches back of the growing tip, the graft being wrapped with raffia and covered with moist cotton. Twenty of 22 such grafts resulted in the development of typical symptoms in the healthy plant, the remaining two grafts failing to unite. In most plants infected in this manner, the first symptoms appeared in the stem to which the mosaic runner was united. Occasionally, however, the first sign of the disease appeared in a runner other than that grafted, indicating a rather rapid passages of the virus down the stem. The incubation period of the virus varied from 14 to 28 days, with most of the plants showing definite symptoms within

20 days. The first symptoms consisted either of the development of small yellow spots on leaves which had not reached full size (Fig. 1, C), or there was a clearing of small segments of the veins similar to that shown in figure 1, B. All the runners of an infected plant eventually were diseased and the later symptoms included all the types shown in figure 1. Ten of the fleshy roots of the infected plants were grown in sterilized soil in the greenhouse and all produced shoots with typical mosaic symptoms.

Attempts were also made to secure infection by cleft-grafting a scion, taken from the tip of a mosaic shoot, on a healthy stem from which 2 inches of the tip had been removed. All of 20 such grafts failed to unite, but in 2 of the healthy plants evidence of infection appeared within 16 days after the graft was made. Since the mosaic scions in these instances had wilted and died within 24 hours, it would seem that the virus must have gained entrance into some tissue of the healthy stem, possibly the phloem, in which it was able to multiply with some rapidity. Attempts have since been made to secure infection by hypodermic injections and by needle pricks made at various points in the ends of freshly cut stems, but so far all have been unsuccessful. Wedges of freshly cut stem tissue inserted in longitudinal slits in the stem also have failed to cause infection.

Transmission by Insertion of Plugs of Mosaic Root Tissues in Healthy Roots

The simplest method of transmitting the virus to sweet potato has been the insertion of plugs from mosaic-diseased roots into roots of healthy plants by the method used by Goss (1) in work with viruses affecting potato [Solanum tuberosum L.]. The healthy sweet potato root was first cut in halves and one portion planted as a control. A core of diseased root tissue was then removed from the fleshy root of a mosaic plant with a cork borer and inserted in a hole made in the remaining half of the healthy root with a borer one size smaller. In many of these experiments actual union occurred between the mosaic root plug and the surrounding healthy tissue but, even when the plug eventually decayed, the shoots from the inoculated roots always developed typical mosaic symptoms. A total of 73 roots were inoculated in this manner and all developed mosaic shoots that did not arise from the cores of mosaic tissue inserted in the roots. All control roots produced only healthy shoots.

Tests for Transmission by Successive Cutting of Diseased and Healthy Roots

All attempts to transmit the virus by successive cutting of diseased and healthy roots have failed. In these experiments the healthy roots were cut in two with a sterilized knife and one portion planted as a control. The other portion was again sliced with a knife that was thoroughly coated with the juices of a freshly cut mosaic root. Thirty-four roots of either Nancy Hall or No. 029878 were treated in this manner but all the shoots from control and inoculated roots remained healthy.

Tests for Transmission Through Fibrous Roots

Soil in which mosaic plants were growing in the greenhouse was removed from the pots and passed through a one-half-inch mesh screen to remove all the larger fibrous roots. Two days later the soil was placed in sterilized 6-inch pots and one-half of a healthy root (Nancy Hall) planted in each pot. The remaining half-roots were planted in pots of steam-sterilized soil to serve as controls. Thirty plants were grown from roots planted in the contaminated soil but no infection was evident during the 90 days the plants were held in the greenhouse. Roots planted in sterilized soil produced only healthy shoots. Thirty healthy roots also were planted in unscreened soil that had grown mosaic plants and then had been stored for 30 days in a moist state in the greenhouse. All of the shoots of the plants of this series remained healthy.

Attempts to infect healthy plants by mechanical inoculation of the feeding roots also have been unsuccessful. The inoculations were made with juices of leaves, feeding roots, and storage roots but no infection occurred in the 24 plants thus inoculated.

Tests for Insect Transmission

No real attempt has been made to determine possible insect vectors of the virus but some observations indicate that it is not readily transmitted by aphids. During the summer of 1944, aphids appeared on runners of healthy and mosaic sweet potatoes growing in immediate contact in the greenhouse. No evidence of infection appeared in the healthy plants during 3 months' observation. Aphid-infested leaves from mosaic plants were removed and placed on leaves of 10 healthy plants, but here again there was no evidence of infection in plants held for 60 days. The aphids, from a superficial inspection, appeared to be *Myzus persicae* Sulz., which was present on peppers in the same house at the time.

Tests for Transmission by Dodder

Attempts have been made to transmit the sweet-potato virus by means of two species of dodder, *Cuscuta arvensis* Beyrich, and *Cuscuta californica* Choisy. Up to the present, however, we have not been able to establish either of these species on the sweet potato, but the experiments are being continued.

CROSS-INOCULATION EXPERIMENTS

Efforts have been made to transmit the sweet-potato virus to the Samsum variety of Turkish tobacco (Nicotiana tabacum L.), N. glutinosa L., Datura stramonium L., cucumber (Cucumis sativus L.), and morning glory (Ipomea purpurea Lam.). All of these plants were inoculated with the expressed juices of mosaic leaves of sweet potato, using the leaf-rubbing method with carborundum powder. The number of plants inoculated varied from 9 to 30. No infection was obtained in any series.

Attempts also have been made to infect sweet potato (Nancy Hall) with the viruses of ordinary tobacco mosaic and the southern celery strain of ordinary cucumber mosaic. Thirty plants have been inoculated with each of these viruses by the leaf-rubbing method, using carborundum powder. No infection was obtained from any of these inoculations.

VARIETAL SUSCEPTIBILITY

No extensive tests of varietal susceptibility have been made, but all the varieties tested have proven susceptible to the virus. These inoculations have been made by the root-plug method, using from 3 to 9 plants of each variety. Infection occurred in all the inoculated plants, the controls all remaining healthy.

The named varieties infected were: Nancy Hall, Porto Rico, Triumph, and Wennop. Varieties received from the Division of Plant Exploration and Introduction under the numbers 129651 and 129653 also were infected, as were seedlings listed as B-219, B-2496, and B-2521. All of these plants showed symptoms of the type described and all appeared to be equally severely affected.

DISCUSSION

Apparently the mosaic disease of sweet potato found at Beltsville is not common in the field. The symptoms are generally so striking that diseased plants are readily noted and, if it were of frequent occurrence, one would expect more reports of it. We have not attempted to determine the distribution of the disease, however, and while the files of the Division of Mycology and Plant Disease Survey contain a number of reports of the occurrence of "mosaic" in various States, it is not possible to say how many of these may have referred to the disease we have studied.

Since the virus is not readily transmissible except by tissue union or prolonged contact of diseased and healthy tissue, it is not likely to be widely disseminated by means other than an insect vector. So far, the lack of evidence regarding such vectors prevents any statement as to the likelihood of its general distribution.

It seems probable that this virus is distinctly different from those whose transmission or other behavior were studied by earlier workers. The symptoms of the mosaic diseases investigated by Rosen, Weber, Weimer, and Harter and Whitney all seem to have been much alike and were essentially different from those of the disease we have studied. The extremely long incubation period reported by Rosen is unlike that of our virus and if, as indicated by the results of Rosen and of Weimer, the causal agent was not transmitted by grafting, there would be much doubt as to whether a virus was actually concerned. It is possible, however, that further attempts with such methods might have produced infection in healthy plants. Wellman's description of the symptoms in sweet potato infected with the southern celery strain of the cucumber-mosaic virus does not correspond with what has been

observed in plants infected with our virus, and the short incubation period that he mentions is a further indication of a difference in the two viruses.

The so-called second type of mosaic described by Weber and West was characterized by a yellowing of the veins that is suggestive of that found in our infected plants. Although the later symptoms of this "second" type were rather different from those produced by our virus, it is not impossible that the two viruses were of a similar type. Since no comparative material is available, however, and since no attempts at transmission of the second virus were reported, it is not possible to make a definite statement as to the relationship of this virus to ours. The same situation exists with the virus reported by Hansford in East Africa although here again there is a possibility that the virus he describes is very similar to the one we have studied at Beltsville.

Since our virus appears to differ both in symptoms and methods of transmission from that described by Rosen under the name of sweet-potato mosaic, it is suggested that it be known as feathery mottle of sweet potato. Under the system of classification of the genera of plant viruses proposed by Mc-Kinney (4) it should be placed in the genus Flavimacula since it is transmitted only by tissue union or prolonged contact of diseased and healthy tissue, and insect vectors are unknown. It is proposed that it be known as Flavimacula ipomeae sp. nov. with the following description.

Flavimacula ipomeae sp. nov.

Common name of disease.—Feathery mottle of sweet potato.

Host reaction.—In Ipomeae batatis L. var. Nancy Hall, and other common varieties of sweet potato, first induces a finely branched yellow mottle along the small veins with or without the development of many small, diffusely margined spots 1 to 2 mm. in diameter. Leaves produced after infection slightly rugose but not much distorted; mottled with areas of light and dark green and showing feathery patches of yellow in dark portions of the leaf. No necrosis of leaves, stem, or roots. Plants considerably dwarfed. Not known on hosts other than sweet potato.

Transmission.—Limited to tissue union by approach grafting of stems or by insertion

of cores of diseased root tissue in healthy roots. No insect vector known.

Physical and chemical properties.—Not known.

Distribution .- Observed only at one locality in Maryland.

SUMMARY

A virus disease of sweet potato causing an unusual type of feathery yellow mottling has been observed at one locality in Maryland. It does not appear to be common in the field. The infected plants are considerably dwarfed but there is no pronounced distortion of the leaves and no necrosis of stems, leaves, or roots.

The virus has been transmitted by grafting of diseased healthy stems and by the insertion of cylindrical plugs of diseased root tissue into healthy roots. No transmission has been secured by other methods of artificial inoculation and no insect vector is known.

The virus produces symptoms distinctly different from those mosaic diseases of sweet potato whose transmission was studied by earlier workers and it is proposed that it be known as feathery mottle of sweet potato.

Under the system of generic classification proposed by McKinney it is suggested that it be listed as Flavimacula ipomeae sp. nov.

BUREAU OF PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND.

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MOVEMENT OF MOSAIC VIRUS THROUGH SUGAR-CANE SEED PIECES

I. L. FORBES AND P. J. MILLS (Accepted for publication April 17, 1945)

In Louisiana, sugar cane is propagated by planting seed pieces, these usually being whole stalks. On each stalk there are usually from 10 to 20 buds or eyes, each of which, if sound, is potentially capable of developing a shoot. In the field, however, even under the best of conditions, rarely more than 20 to 25 per cent of the buds produce shoots which survive. For these seed pieces, the term "seed cane" is ordinarily used.

Usually when a mosaic-diseased seed piece is planted, all the shoots growing from it have the mosaic symptoms. Conversely, mosaic-free seed pieces produce only mosaic-free shoots. To have available mosaic-free plants for inoculation purposes, mosaic-free seed pieces are planted either in the greenhouse or in isolated places in the field. The chance of such plants picking up the infection during the early stages of growth is very slight.

During the winter of 1941–42, while conducting some inoculation tests in the greenhouse on young shoots of two varieties, Co. 281 and C.P. 28/19, which had grown from mosaic-free seed pieces, it was observed that not only did the plants that were inoculated develop the mosaic symptoms, but mosaic developed in some other shoots close by which were not inoculated. When the latter shoots were removed from the soil it was found that they were attached to the same seed pieces as were the inoculated shoots. Since there was no evidence of what could have been interpreted as natural infection, i.e., transmission by insects, in other cane growing in the greenhouse, the possibility of virus movement through the old seed piece was suspected. Following this, field inoculations were made to determine whether or not there may be a transfer of the virus through the seed piece, and, if so, what significance is to be accorded such a method of mosaic spread in sugar-cane fields. These tests were made during three growing seasons.

All tests were carried through in a similar way. Full-length stalks were planted and then a single shoot developing from each seed piece was inoculated by placing a few cc. of extracted juice from a mosaic-diseased plant in the whorl of leaves at the top of the plant, then pricking through the spindle a number of times with fine needles. When shoots from other nodes showed the mosaic symptoms, the seed pieces were dug and the presence or absence of mosaic was recorded for all shoots which had developed from the different nodes.

Young shoots developing from buds on the nodes sucker freely and consequently instead of a single plant there is soon a cluster of plants, commonly called a stool, growing from a node. Usually the mosaic will spread rapidly from one plant to the others in a stool. In the following discussion the term "shoot" is used indiscriminately for a single plant or one among the many in a stool.

Tests in 1942. Data were obtained from three inoculation experiments. Two varieties, C.P. 28/19 and C.P. 28/70 were used. A total of 50 plants were inoculated; 29 of them developed mosaic. Virus spread, apparently, from 10 of these inoculated plants to noninoculated plants.

From the standpoint of the presence or absence of mosaic, the condition of all the shoots on these seed pieces in which there appeared to be a movement of the virus is shown in table 1. It will be observed that the virus spread from the inoculated shoots in both directions in the seed pieces, but in some cases passed by certain nodes and infected shoots further removed from the ones inoculated. In figure 1 is shown one of the seed pieces (test A, seed-piece No. 9) at the time it was removed from the soil.

TABLE 1.—Spread of virus in seed pieces, 1942; based on occurrence of mosaic in shoots on nodes other than the one at point of inoculation

	α		Nodes numbered from point of inoculation									
Test	Seed piece		Bas	sipeta	etal spread			Inoculated	Apical spread			
	number	6	5	4	3	2	1	shoot	1	2	3	
A	 2		+	0		+	+	I				
	3 9	+		++	+	+	4	Ï			,	
В	2				,	+		Ţ		******		
	6 18	+	*****	+	,,,,,,	+	+	Ī	0	0	0	
$^{\prime}$ C	9	0	0	+	0	0	+	Ĩ	0	+	+	
	17 19	****** ******	******	+	+	+	+	I		0	,,,,,,	

Symbols: I = Inoculated shoot.

+= Shoot with mosaic.

0 = Shoot with no mosaic symptoms.

On nodes without symbol, shoots not present.

Tests in 1943. Data were secured from four tests in 1943. The varieties C.P. 28/70 and Co. 281 were used. A total of 55 Co. 281 plants was inoculated. Mosaic developed in 17 of these. Virus spread from 6 inoculated plants to a total of 9 other shoots in 6 of the 17. With C.P. 28/70, a total of 45 plants was inoculated; 18 of them developed mosaic. Virus spread from 8 of the inoculated plants to a total of 14 noninoculated plants, apparently through the seed pieces.

Tests in 1944. In 1944, data were secured from 4 tests, 2 with the variety Co. 281 and two with C.P. 28/70. Two of the tests (A and B) were started in May and 2 (C and D) in June. As the results in general were very similar to those obtained in preceding years, they have been summarized in table 2. In all the tests mosaic was observed on some shoots that were not inoculated, apparently due to the transference of the virus through the old seed pieces. The virus seemed to travel in the seed piece equally well in either direction, some of the infected shoots being toward the base from the

inoculated one and others toward the top. In some cases, also as in previous years, the virus passed by some of the nodes without entering the shoots and infected others further along the stalk.

In table 2 the shoots under the heading "with mosaic" include the inoculated shoot. In all seed pieces, then, on which there was observed more than one shoot with mosaic, it is assumed that there was a transference of the virus from the inoculated shoot to the others. In test A, such a transference occurred in 14 out of 20 seed pieces; in test B, 13 out of 19; in test C, 6 out



FIG. 1. There were 9 buds (eyes) on the seed piece. Plants developed from buds 2, 4, 7, 8, 9. Plant 8 was inoculated. Mosaic developed in the inoculated plant and its suckers and also in plants from nodes 2, 4, and 7. Plants from node 9 remained mosaic-free.

of 21; in test D, 3 out of 25. Possibly the lower percentages in the two later tests can be explained by the greater deterioration in the seed pieces at the time the shoots were inoculated.

DISCUSSION

Sugar-cane mosaic is a virus disease known to be transmitted from plant to plant principally by insects. Infection is brought about naturally by certain insect vectors which suck the infectious juice from mosaic-diseased plants and then transmit it by feeding on healthy plants. In artificial inoculation experiments the commonest method of virus transfer is the injection of extracted juice from mosaic-diseased plants into the spindle of growing plants. Symptoms appear in the new growth usually within ten days to

three weeks. The rate of growth of the sugar-cane plants seems to determine time elapsing between inoculation and the appearance of the symptoms. Rapidly growing plants develop mosaic more quickly than those growing slowly.

In sugar-cane plots planted with mosaic-free seed cane and well-isolated from mosaic-diseased sugar-cane or corn, very little mosaic develops from natural infection, especially during the early growing season, which in

TABLE 2.—Movement of mosaic virus through sugar-cane seed pieces. One plant per seed piece inoculated

	Test A, C.P. 28/	770 Test B, C	o. 281	Test C, C.	P. 28/70	Test D	, Co. 281
Sced piece	Number of shoo	ots Number of	shoots	Number o	f shoots	Number	of shoots
number	With Mosa mosaic free		Mosaic- free	With mosaic	Mosaic- free	With mosaic	Mosaic- free
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 Total	3 1 3 1 4 1 1 1 1 2 0 3 0 2 0 2 1 3 0 4 1 3 0 7 0 2 1 0 3 1 5 4 0 1 1 1 0 1 0 5 1	2 3 1 3 1 4 3 1 1 2 1 2 3 1 2 3 3	0 0 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	3 1 1 1 1 2 1 1 1 1 2 1 1 1 1 1 1 1 1 1	3 1 1 3 1 2 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 2 0 0 1 1 2 0 0 1 1 1 1	1 2 2 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 2 2 2 2 0 0 1 0 0 1 1 0 0 0 1 0 0 0 0

Louisiana extends into early June. Consequently, when it was observed that plants other than those inoculated developed mosaic, virus transfer through the seed piece was suspected. This suspicion was strengthened by the fact that no natural infection was found in the same varieties in other adjacent parts of the field where no artificial inoculations had been made. To confirm this, inoculation experiments were made in the field during three seasons. These were so designed as to give results that would answer the question as to whether sugar-cane mosaic can spread from plant to plant through the seed piece. The results seem to justify the conclusion that such a method of mosaic spread in sugar cane in Louisiana not only does occur but that it may occur quite commonly. The virus may travel from the point of inoculation either toward the top or toward the base of the seed piece.

Of particular interest, physiologically speaking, is the fact that the virus passes through the seed piece, skipping some plants but infecting others further removed from the point of inoculation. The virus apparently did not travel through all the seed pieces. This perhaps may be explained by the fact that portions of the stalks often disintegrate during the winter and spring seasons before or after the development of the young shoots. It is not probable that the virus would spread through dead or injured stalk tissues.

Whether under ordinary field conditions there is a transference of the virus through enough of the seed pieces to be of significance has not been determined. It is known, however, that mosaic spreads more rapidly in plant cane (plants from seed pieces) than in stubble cane (plants from rootstocks remaining in soil after harvest). There is a possibility that the greater spread in plant cane as compared to stubble cane may be due, in part at least, to the transfer of virus through the seed pieces from infected to noninfected plants.

SUMMARY

Sugar cane is propagated vegetatively by planting whole stalks or portions of stalks. Usually a number of shoots develop from each seed piece.

From inoculation experiments, it has been found that the mosaic virus can spread from an infected shoot through the old seed piece to other shoots. The virus spreads through the seed piece in either direction. The virus can also pass by a node and infect other shoots further removed from the point of infection.

DEPARTMENT OF PLANT PATHOLOGY,
LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LOUISIANA.

RHIZOCTONIA BUD ROT OF STRAWBERRY PLANTS

J. B. DEMAREE 1

(Accepted for publication May 12, 1945)

In 1935 Brooks² reported on a bud rot of strawberries in Florida and attributed it to a *Rhizoctonia* of the *solani* type. He described the disease as a "dry rot of leaf- and flower-buds, and bases of petioles and stipules, the last causing the older leaves to lose their upright position and lie flat on the ground." Brooks isolated the fungus and was able to reproduce the disease in the greenhouse.

What appeared to have been the same disease has been observed by the writer during the past few years in Arkansas, Delaware, Maryland, Mississippi, North Carolina, and Tennessee. Bain^{3,4} reported observations on the disease in Louisiana and Mississippi in 1944.

This disease shows best in the field for a few weeks after winter dormancy has been broken and the plants have resumed growth of the central cluster of leaf and flower buds. In Florida this period occurs in December and January, in southern Louisiana and Mississippi in February and March, in Arkansas and Tennessee in March and April, and in Delaware and Maryland in April and May.

The first sign of the disease is an apparent retardation in growth of crown buds of infected plants as compared with normal ones. Later both leaf and flower buds turn brown and die, and are easily lifted out of their position. Simultaneously the outer previous year's leaves assume a more or less horizontal position and become darker green than leaves of normal Several adventitious leaf buds soon develop under the residue of the originally killed buds and these may likewise be killed, or a few may survive but produce at first a weak, spindly growth, and later the usual form of "multiple crown" plants. Sometimes plants are killed outright. Usually, however, infected plants recover but produce no fruit that year. In dissecting the crown of an infected plant, one finds that buds have large, soft lesions or that they are partially or completely disintegrated. Another symptom of the disease, which is an after-effect of the early stage of bud disintegration, is that some leaves of surviving buds are injured before they unfold and after expanding are necrotic at the tip and margin of the leaflets. Sometimes an entire leaflet is missing. The effect of this injury persists throughout the life of the leaves. The dead leaf tissues slough off, leaving misshapen, puckered, or crinkled leaves. Brooks² has observed that stolons

² Brooks, A. N. Rhizoctonia bud rot of strawberry. (Abstr.) Phytopath. 25: 965.

Agr., Plant Dis. Reptr. 28: 259. 1944. U. S. Dept.

¹ Senior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Md.

³ Bain, Douglas C. Strawberry diseases in Mississippi. U. S. Dept. Agr., Plant Dis. Reptr. 28: 165. 1944.

and fruits are also attacked by the same fungus that causes bud decay. The stalks and root systems of strawberry plants are not attacked by the fungus, and this bud rot should not be confused with a Rhizoctonia root disease reported by Zeller⁵ as causing extensive damage to strawberry roots in Oregon.

As a rule, the disease is of slight importance in strawberry fields, affecting only a few widely scattered plants. It is absent from many fields, but occasionally causes considerable damage. The most the writer has seen occurred in a single field near Van Buren, Ark., in which 25 or 30 per cent of the plants were estimated to be infected. Bain³ observed the disease near Meridian, Mississippi, in 1944 and reported that in one field fully 30 per cent of the plants were infected. It is not known why the disease may become epiphytotic in some fields and be absent from others. It has been observed, however, that plants seem to be predisposed to infection when they are partially covered with wind-blown sand or soil, or when flood water has deposited some soil around the plants in low parts of a field.

The fungus is thought to be one of the variants of *Rhizoctonia solani*. When grown upon corn-meal agar it forms reddish-brown, loose, aerial hyphae with numerous black sclerotia. The sclerotia, in an early stage of development, are small aggregates of loosely constructed, short-celled, white hyphae, but later turn brown and become more compact. In the final stage they are globose to flat, dark brown to black, and from 0.5 to 1.25 mm. in diameter. They may form on the surface of the agar, on the inside wall of the culture tube, or among the aerial hyphae. The perfect stage of the fungus has not been seen on strawberry plants nor on artificial media.

Rhizoctonia bud rot may easily be mistaken for other strawberry diseases occurring during early spring. A. N. Brooks writes (correspondence), "It appears that the Rhizoctonia bud rot of strawberry can be confused with several things. Before I discovered the true cause I thought that it was the work of the sucking insect Pamera, which are numerous at times in Florida." The strawberry crown-rot disease, described by Plakidas⁶ from Louisiana and caused by Sclerotinia sclerotiorum, is superficially similar to bud rot and not easily distinguished by field examination unless the black sclerotia of the Sclerotinia are found among the dead crown buds. Bain, in discussing the similarity of the two diseases, said, "This disease (Rhizoctonia bud rot) is very much like the Sclerotinia crown rot, but there is no damping off of basal leaves, nor are sclerotia present on or around the plants."

The spring dwarf disease, caused by the nematode Aphelenchoides fragariae, may also be mistaken for Rhizoctonia bud rot. Both diseases appear at the same time of year, and the symptoms are very similar during the initial stage, i.e., both retard the new spring growth of leaves and flower development. The symptoms of the two diseases are more easily distinguishable at a later period. As a rule, the dwarf disease kills only the

⁵ Zeller, S. M. A strawberry disease caused by *Rhizoctonia*. Oreg. Agr. Expt. Stat. Bull. 295. 1932.

⁶ Plakidas, A. G. Factors responsible for the small strawberry crop this year. U. S. Dept. Agr., Plant Dis. Rptr. 19: 132-133. 1935.

TABLE 1.—Results of inoculating strawberry plants with Rhizoctonia

No. plants	Treatment	Results
6	Uninoculated, crowns exposed	None infected
6	Inoculated, crowns exposed	All infected
6	Uninoculated, crowns covered with soil	None infected
6	Uninoculated, crowns covered with sand	None infected
6	Inoculated, crowns covered with sand	All infected

flower buds. The new leaves, however, are abnormal in appearance and development, because of the injury done to them by the great number of nematodes that have fed upon them during the early bud stage. The result of the nematode injury shows later as distorted, reddish leaves with short petioles and small, narrow leaflets.

Rhizoctonia attacks plants in no pattern of regularity, grouping, or segregation. There may be one or a few bud-rot-infected plants among a group consisting of an original set plant and its family of runner plants, or single bud-rot plants may be widely scattered. In contrast to the irregular distribution of Rhizoctonia bud rot, plants affected with nematodedwarf disease are almost invariably grouped, because the nematodes spread from the infested mother plant to several or all of its daughter plants.

When this bud-rot disease was found in 1942 on the U. S. Plant Industry Station Farm at Beltsville, Maryland, work was initiated to determine the cause of the trouble. It was suspected that either Sclerotinia or Rhizoctonia would be found. Cultures were made and diseased plants were held in moist chambers at different temperatures. Sclerotinia never developed by any method tried, nor were sclerotia ever found associated with the disease in fields. A Rhizoctonia was isolated more frequently than any other one organism. In 1944 the pathogenicity of this fungus was determined in greenhouse experiments. Brooks' findings in 1935 were confirmed.

In a preliminary test started March 4, 1944, 30 plants were set in 6-inch pots filled with unsterilized composted soil. The plants were inoculated by



Fig. 1. The two end plants were inoculated with a culture of Rhisoctonia. The normal plant at center was not inoculated,

placing a small piece of agar containing hyphae and sclerotia of the fungus immediately under the surface of the soil and near the crown of the plants. Table 1 shows the arrangement of the test and the results.

The symptoms of infected plants were essentially identical with those of field-infected plants (Fig. 1). The crowns of a portion of the plants were covered with soil or sand, to determine if covering alone would cause abnormal growth of the plants similar to that caused by the *Rhizoctonia*. The covered plants developed slowly and irregularly, but the leaves finally emerged and plants were normal.

In April, 1944, a second greenhouse test was set up to demonstrate the association of the *Rhizoctonia* with strawberry bud rot. One hundred strawberry plants taken from the field were set in 6-inch pots so that the crown buds were slightly above the soil surface. Sixty-three of the plants were inoculated, the remainder were held as controls. At the termination of the test 30 days later, none of the 37 controls had any evidence of infection. Of the 63 that were inoculated, 40 had typical bud-rot symptoms.

This Rhizoctonia bud-rot disease of strawberries can and does occasionally cause a material loss to the berry crop in some fields. The motive for preparing this paper, however, was not to emphasize the economic importance of the disease, but principally to point out to those interested that there are other diseases of this crop occurring simultaneously and with almost identical symptoms that may easily be mistaken for bud rot. This paper also indicates the known distribution of the disease.

SUMMARY

A bud-rot disease of strawberries caused by a *Rhizoctonia* of the *solani* type, first reported by Brooks from Florida in 1935, was later found in Arkansas, Delaware, Maryland, Mississippi, North Carolina, and Tennessee.

The fungus attacks and kills the flower and leaf buds during a few weeks when buds resume new growth. This period embraces December and January in central Florida, February and March in southern Louisiana, and April and May in Maryland.

The early symptoms of the disease are very similar to and can easily be mistaken in the field for some other strawberry disorders, namely, injuries caused by the sucking insect *Orthea vincta* in Florida; the crown-rot disease caused by *Sclerotinia sclerotiorum* in southern Louisiana; and spring dwarf, caused by the nematode *Aphelenchoides fragariae*.

PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND.

FUNGICIDES IN RELATION TO SCAB AND FRUIT RUSSET OF PEAR IN THE HOOD RIVER VALLEY, OREGON¹

J. R. KIENHOLZ² AND LEROY CHILDS³

(Accepted for publication June 2, 1945)

Some pear varieties must be practically russet-free to command top prices on the market. This is particularly true for Anjou and Comice, two of the choice winter varieties grown in Oregon. While several conditions are known that cause smooth-skinned pears to become russeted, spray russet is one of the common causes for lowered cash returns from the crop.

Pear scab (Venturia pyrina Aderh.) has been in Oregon for many years, but it has been especially troublesome in the main pear-growing districts since 1932 (7). Lime-sulphur, and some of the other relatively efficient types of sulphur may give good commercial control of scab, but they may severely russet the fruits of some varieties and may injure any variety if followed by oil-containing sprays within 45 days. No really satisfactory fungicide has been available in the past that would adequately control scab without producing considerable russet on spray-sensitive pear varieties during most seasons. The present paper reports attempts during the past 12 years to find a satisfactory fungicide for use on Anjou pears in Oregon.

OBSERVATIONS ON RUSSET OF PEAR FRUITS

The origin of various types of fruit russet can sometimes be stated with certainty, but more often, the causes are difficult to determine, particularly in midseason or later.

Several conditions that are known to cause russet on Anjou pears when present at one stage or another in fruit development are: 1. Injuries due to insects, including blister mite, thrips, European red mite, and rust mites. 2. Mildew infection. 3. Environmental factors—excessive moisture and frost injury at various stages of development. 4. Inherent factors—bud sporting and phases of the black-end trouble of pears on Oriental rootstocks. 5. Mechanical injuries. 6. Spray injuries. Frost damage has affected unopened buds during 3 of the past 12 years. The frost russet injury, except when frost rings develop on the fruit, is difficult to distinguish from that caused by certain fungicides or other agents.

Many of the spray materials tested during the past 12 years have caused a definite russet on Anjou pears and had to be eliminated from further consideration. Spray injury is more likely to occur early in the season, the

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United States Department of Agriculture.

² Associate pathologist, Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

s Superintendent, Hood River Branch Experiment Station of the Oregon State College.

sensitive period lasting until after the pubescence has been shed from the young fruits and the hair-attachment injuries have healed. This process is generally completed by the middle of June. After this it is possible to use with safety some of the sprays causing early injury, but it is then too late for efficient scab control.

EARLY SPRAY TESTS

Trees receiving no spray for the entire season often produced fruit with considerable russet injury. In many cases the russet appeared to result from infestations of blister mite and other insects that respond to control by dormant lime-sulphur applications. It was also discovered that the scab fungus overwintered as twig infections (8) which were important in causing primary infections some seasons. Since lime-sulphur was effective in inactivating the open scab lesions in early spring (8), check trees as well as other trees in a plot received a delayed dormant application of lime-sulphur or lime-sulphur-oil (2, see p. 6). This practice gave more uniform results in the evaluating of fungicides by eliminating some of the insect pests capable of causing a fruit russet, and by giving all trees in a plot a nearly equal chance for early scab infection. Lead arsenate at the regular 3 to 100 rate was combined with each test fungicide for the calyx application, and at least one additional application of lead arsenate was made later in the season for codling-moth control.

Spray materials tested for pear scab control may be conveniently classified into 3 general groups: 1. Those that caused definite russeting on Anjou pears, but otherwise might be efficient fungicides. 2. Materials that commonly cause little fruit injury, but gave poor scab control. 3. An intermediate group giving fair or good scab control without causing excessive fruit russet during most seasons. The fungicides falling in each group are

listed below:

Group 1. Fungicides usually causing russet on Anjou pears: Basic copper sulphate, Bordeaux mixture in various combinations, copper-Bordo, copper oxide (red), copper oxalate, copper silicate, copper-hydro "40," copper oxychloride, copper acetonate, lime-sulphur in various combinations, dry lime-sulphur, ammonium polysulphide, flotation sulphurs, colloidal sulphurs, gas-house sulphurs, and micronized wettable sulphurs.

Group 2. Fungicides giving poor scab control: Copper oxide (black), phenothiazine, zinc-Bordo, zinc sulphate, 66A, fused bentonite sulphurs, and

wettable sulphurs coarser than 325-mesh.

Group 3. Fungicides in general use or having commercial promise for scab control on Anjou pears: Wettable sulphurs of approximately 325-mesh, copper phosphate mixture, and ferric dimethyldithiocarbamate (Fermate).

Only copper phosphate mixture, of the fungicides tested up to 1941, compared favorably with the wettable sulphurs, which have been standard for scab control on Anjou pears. The copper phosphate-lime-bentonite mixture (3) was used at a 4-8-4-100 ratio in earlier tests, and later at 4-4-4-100 to reduce some of its bulk. The wettable sulphurs, with the exception noted

TABLE 1.—Scab and russet in jury on Anjou pears in a single orchard for 7 years

	Cover	Check	trees	Copper	phosphate	Wettabl	Wettable sulphurb		
Year	sprays	Scab	Russet	Scab	Russet	Scab	Russet		
	Number	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent		
1935	4	30.4	1.2	2.4	2.0	4.1	2.5		
1936	$\tilde{3}$	9.0	6.9	0.8	3.3	0.8	20.1		
1938	4	32.5	18.9	1.3	13.8	1.9	86.0		
1939	2	0.3	50.0	0.0	26.0	0.2	51.2		
1942	4	56.4	5.3	8.6	8.4	9.9	86.8		
1943	3	79.2	9.0	21.3	4.0	47.2	37.9		
1944	3	79.3	11.1	19.7	8.6	10.3	68.1		
Avera	ıge	41.0	14.6	7.7	9.5	10.6	50.4		

^a Injury sufficient to lower fruit grade from extra-fancy to fancy or lower by Western grading rules.

b Wettable sulphur of 325-mesh except in 1942 when micronized wettable sulphur was used.

for 1942, were commercial brands of approximately 325-mesh fineness used at 8 or 10 pounds per 100 gallons. Commercial spraying equipment was used in all tests. The use of the two fungicides on Anjou pears for 7 seasons in the same orchard gives a fair comparison of their effectiveness in scab control and the degree of grade-reducing russet caused by them (Table 1).

In this series of plots copper phosphate gave about the same control of scab as wettable sulphur, except in 1943, when the control was much greater with copper phosphate. Neither copper phosphate nor wettable sulphur has given satisfactory scab control when the unsprayed check trees have produced approximately 50 per cent or more infected fruits. The distinct advantage of copper phosphate over wettable sulphur under average conditions has been the production of a greater amount of russet-free fruit.

In orchards where scab has been more severe, or when the spray schedule has not been well timed, copper phosphate has controlled scab better than wettable sulphur. The relative merits of the two fungicides in heavily infected orchards are shown in table 2. Observations in the test plots and in commercially sprayed orchards indicated that the fungicidal activity of wettable sulphur sprays was largely dissipated within 10 to 15 days after

TABLE 2.—Scab control by copper phosphate and wettable sulphur in heavily infected Anjou pear orchards

Year	Cover	Checl	trees	Copper p	hosphate	Wettable sulphur		
хеаг	sprays	Scab	Russet	Scab	Russet	Scab	Russet	
	Number	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
1933	2	99.4	49.8	72.6	63.0	96.0	81.8	
1934	4	82.6	96.7	13.6	94.8	63.1	100.0	
1937	3	99.5	yattarist.	38.2	**********	67.8		
1940	3	96,9	63.0	26.7	79.1	53.5	68.9	
1941	4	96.8	4455+4544	21.3	increpate .	47.2	8,,,,,,,	
Avera	ze	95.0		34.5	а	65.5	a	

^{*} Cause of fruit russet obscured by frost damage and other factors.

application, whereas the residue of the copper phosphate mixture was more resistant to weathering and retained its activity longer.

Pears sprayed with copper phosphate have generally produced a smoother skinned fruit than those in any other test plot. The degree of smoothness was much greater than the figures in table 1 indicate. An interesting characteristic of the copper phosphate mixture was its ability to decrease russet injury, even though russeting was obviously due to frost damage, as in 1939 (Table 1). Less fruit russet often occurred in the copper phosphate plots than on unsprayed pears. The reasons for the decreased russet injury in such cases are not understood. On the other hand, in orchards remaining wet for extended periods or during abnormally wet seasons, definite spray russet has resulted from the use of copper phosphate. Results for 1938 and 1944 in table 1, and, as an extreme example, for 1933 in table 2, are illustra-

TABLE 3.—Effect of wet and dry locations in the production of fruit russet on Anjou pears

Classic and the color and	Cover Year		Total fru	Total fruit russet			
Spray treatment	sprays	tested	Wet orchard	Dry orchard			
Name and the second	Number		Per cent	Per cent			
Copper phosphateWettable sulphur		1938 1938	47.1 99.4	$\begin{array}{c} 0.04 \\ 2.3 \end{array}$			
Unsprayed trees Copper phosphate Wettable sulphur	2	1939 1939 1939	50.0 26.0 51.2	$7.2 \\ 0.0 \\ 0.4$			
Unsprayed trees Copper phosphate Wettable sulphur	3	1940 1940 1940	63.0 79.1 68.9	$8.4 \\ 12.4 \\ 14.2$			

tions of injury caused during wet seasons or in wet orchard locations. The actual percentages for fruit injury in table 1 are not great enough to give a true picture of the injury. In recording the data for 1944, in particular, a good share of the russet injury was on the border line of commercial damage. During most seasons, however, the russet on copper phosphate-sprayed fruit was so slight that the fruit could almost be classed as russet-free. It seems probable that the lime-bentonite mixture is able to neutralize the free copper released from copper phosphate under average weather conditions. With more extreme and extended wet periods, the liberated copper that is constantly present apparently becomes toxic to the fruit skin.

The orchard environment exerts a strong influence on the severity of fruit russet caused by the action of fungicides. Excessive moisture in the form of rain, dews, fog, or very high humidity increases the toxic effects of the fungicides tested. These same factors also favor an increase in scab infections. For convenience of comparison the test plots were classed as wet or dry orchard locations; and the effect of fungicides in causing fruit russet during the same years in similar spray schedules, but in radically different environments, is illustrated in table 3.

The early spray tests showed that copper phosphate gave more effective scab control and less russet on Anjou pears than wettable sulphur. A fungicide more potent against pear scab and less likely to cause injury during wet seasons was still desired for use on spray-sensitive pear varieties.

RECENT SPRAY TESTS

Several of the new organic fungicides offer considerable promise for use on pear trees. For the past 3 years ferric dimethyldithiocarbamate (Fermate) (4) has shown excellent possibilities as a fungicide for pear scab control on the Anjou variety. Results of its use in the same orchard for the 3 seasons are compared with those for other fungicides in table 4.

TABLE 4.—Control of scab and fruit russet on Anjou pears in the same orchard for 3 years

	19)42	19	43	1944	
Spray treatmenta -	Scab	Russet	Scab	Russet	Scab	Russet
	Per cent	Per cent	Per cent	Per cent	$Per \\ cent$	Per cent
Check trees—No cover sprays Copper phosphate 4-4-4-100 Wettable sulphur, 325-mesh, 8-100	56.4 8.6	5.3 8.4	79.2 21.3 47.2	$9.0 \\ 4.0 \\ 37.9$	79.3 19.7 10.3	11.1 8.6 68.1
Wettable sulphur, micronized, 8-100 Fermate 1½-100	9.9 3.8	86.8 2.0	36.0 8.0	50.2 0.6	10.3 5.0	71.0 3.6

^{*} Four cover sprays applied in 1942; 3 each in 1943 and 1944.

Fermate has given better scab control and caused less injury to Anjou pears than any of the fungicides tested. It has been particularly beneficial during the past 2 seasons when scab infections have been relatively numerous. The Fermate deposit appears to be so persistent and active against the scab fungus that it seems especially desirable to combat early season infections.

The addition of lime to Fermate sprays decreased the value of Fermate for scab control by nearly half during 2 years, and a peculiar ring-spot injury formed in young leaves. While no permanent injury could be detected on tree or fruit, the combination is considered undesirable, especially since lime decreases the persistence of the spray deposit.

Bartlett pear leaves often become more heavily infected by the scab fungus than Anjou leaves, and since these are active infection sources, more fruit infections may develop on Bartlett than on Anjou. On the other hand, Bartlett pear fruit is not very susceptible to spray injury and a russet or other injury not deforming the fruit, does not affect the grade for cannery use. It is therefore possible to use stronger and more effective scab sprays on Bartlett and other spray-tolerant varieties. The comparative value of 3 fungicides in Bartlett pear scab control in a heavily infected orchard during 1944 is shown in table 5. Lime-sulphur was the most effective spray in controlling scab in the heavily infected orchards and probably actually "burned out" many active scab lesions.

TABLE 5.—Scab control in a heavily infected Bartlett pear orchard during 1944

Spray treatment (in 3 cover applications)	Fruit scab	Leaf scab	Fruit russet injury
	Per cent	Per cent	Per cent
Check trees—No cover sprays	94.9	83.0	0.0
Fermate 1½-100	25.5	41.9	0.0
Bordeaux 4-4-100	12.2	11.5	37.2
Lime-sulphur 3-100 in Pink; 2-100 in 2 cover sprays	8.9	7.2	0.0

THE SPRAY SCHEDULE

The number of sprays that must be applied to control pear scab will vary according to weather conditions and the amount of scab present in the orchard. Most fungicides are applied to prevent infections rather than to kill the fungus after it becomes visible on the fruit or leaves. If scab is prevented from developing early in the season, or is held to a small amount, the chances for good commercial control for the remainder of the season are greatly increased. A single spray application may mean the difference between a clean or scabby crop. Micronized wettable sulphur 8 to 100 was applied to pear trees during 1943 and 1944 in a partial schedule. The value of the different spray applications in scab control on Anjou pears during these years is shown in table 6. While 3 applications, pink, calyx, and first cover, gave the best control, they also caused by far the most fruit russet.

Timing is also an important factor in successful scab control. Scab control was poorer during 1943 than in 1944 with the same fungicide, even though unsprayed trees developed almost identical amounts of scab during both years (Table 6). Infections resulted in 1943 when spray coverages were largely dissipated during several rains. During 1944, infection periods occurred only just after sprays had been applied, thereby allowing the spray deposits to exert their maximum influence.

After a good spray material has been properly timed, it is still necessary to apply the fugicide thoroughly. More attention should be directed to applying a large share of the fungicide to the tree tops. It is there that scab control is poorest (1), and a good spray deposit there will give

TABLE 6.—Scab control and fruit injury resulting on Anjou pears from various applications of wettable sulphur

	19	43	1944			
Spray applications applied	Scab	Russet	Scab	Russet		
	Per cent	Per cent	Per cent	Per cent		
Check trees-No cover sprays	79.2	9.0	79.3	11.1		
Pink spray only	63.8	14.5	47.3	33.4		
Calyx spray only	70.0	21.4	59.4	50.2		
Pink and calyx spray only		21.3	26.0	47.6		
Pink, calyx, and first cover spray	36.0	50.2	10.3	71.0		

considerable protection against infections by redistribution to unprotected parts of a tree during subsequent rains (5). The spray nozzle pressure should be cut down on high-pressure equipment so that only a "fog" is applied to the lower parts of the tree. This is particularly important when dirty water or coarse particles of spray material are in the spray tank. Specific data are lacking, but both writers have frequently observed that considerable russet may be caused on tender-skinned pears by driving fine particles of silt or coarse spray particles against the fruit surface under high pressure. The relation between scab control and injury caused by wettable sulphur (8 to 100) on Anjou pears at various heights in the tree is shown in table 7.

TABLE 7.—Relation of the height of fruit on the tree to scab control and russet injury on Anjou pears following wettable sulphur spray

Fruit locations	Scab	Russet
	Per cent	Per cent
Bottom third of tree	 10.0	62.0
Middle third of tree	 14.5	27.3
Top third of tree	23.2	16.9

a Trees averaged approximately 25 feet in height.

EFFECT OF FUNGICIDES ON FOLIAGE COLOR AND FRUIT SET

It was noticed during the early spray tests that sulphur fungicides usually caused Anjou pear leaves to remain yellower throughout the season than leaves on trees sprayed with other materials. A number of growers also suggested that the use of sulphur was decreasing their yield of fruit. Tests were started in 1942 to evaluate these observations. The data are not complete enough to make final statements, but certain trends requiring closer study have been indicated.

Trees sprayed with copper phosphate or with Fermate and those left unsprayed developed distinctly greener foliage than sulphur-sprayed trees. These reactions have been consistent during 3 years, although the degree of color difference has varied somewhat with the seasons. The finer sulphurs appear to be more toxic to the trees than the coarser forms, but the orchard environment, rootstocks, and other factors probably influence the extent of injury. Leaf color has been improved most in the copper phosphate plots. Kadow and Anderson (6) reported a similar reaction in cherry trees after the use of copper phosphate.

Large differences in the average fruit yield occurred on the test trees in 1942. Trees sprayed with micronized wettable sulphur produced only about 1/3 as much fruit as the check trees or those sprayed with copper phosphate or with Fermate. The total blossoms were counted on test trees during 1943 and 1944 and the actual fruit set was determined from the total fruits harvested. The crop in 1943 was too light to provide reliable information. In 1944, the set of fruit varied too greatly among trees with

the same treatment to permit definite conclusions, but again the micronized sulphur seemed to reduce the set of fruit below that of the other plots.

SUMMARY AND CONCLUSIONS

A spray material that is more effective against pear scab and less toxic to Anjou and other spray-sensitive pears has been needed to replace the wettable sulphurs now used because of the lack of better fungicides. A fungicide is often needed for scab control during the summer, but sulphur materials have 4 evident disadvantages for use on Anjou pears during that 1. They may cause a severe sun-scald type of injury on fruits when temperatures rise above 90° F. 2. Insecticides containing oil may not be applied within 45 days after sulphur fungicides on pear trees for fear of causing severe leaf-spotting or defoliation. This fact seriously hampers the control of late-season spider mite attacks in scabby orchards. 3. Sulphur is dissipated rapidly during warm weather and is effective in scab control for only relatively short periods. 4. Sulphur apparently is directly toxic to Anjou pear trees.

Lime-sulphur was the most satisfactory fungicide tested for pear scab control on those varieties not subject to spray injury.

Copper phosphate has given equal or better scab control than wettable sulphur in most cases, and caused much less fruit russet during average weather conditions. During wet seasons it has not given adequate scab control and it produced excessive fruit russet. The appearance of foliage sprayed with copper phosphate has been superior to that in any other spray plot. This material has not reduced fruit set and has been compatible with most insecticides. It has not been available during the war, and the mixture is so bulky that growers object to it.

Fermate seems to be an immediate substitute for the wettable sulphurs for use on spray-sensitive pear varieties. It has given consistently good scab control and has not injured fruit for the past 3 years, and foliage color has been excellent on sprayed trees. Fermate has given no trouble in most mixtures and oil may be safely used in the same application or soon afterwards.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES, U. S. DEPARTMENT OF AGRICULTURE, AND OREGON STATE COLLEGE.

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THE RELATION OF THE OCCURRENCE OF FOLIAGE SYMPTOMS OF CHLOROTIC STREAK OF SUGAR CANE TO THE DISTRIBUTION OF THE VIRUS IN THE PLANT

E. V. ABBOTT1

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INTRODUCTION

Foliage symptoms of chlorotic streak of sugar cane are inconstant. Although some infected plants may manifest them throughout their period of growth, frequently the characteristic leaf streaks are present only intermittently. There is little information in the literature as to the relation of this inconstancy of foliage symptoms to the occurrence of the virus in the stalk or to actual recovery from the disease. Whether, for example, the foliage "recovery" resulting from the senescence of old, streaked leaves, and the absence of streaks on the new foliage subsequently produced, is related to disappearance of the virus from the stalk, has not been previously investigated. A knowledge of these points is of practical value in studying control measures, as well as from the standpoint of gaining a better understanding of the disease itself, and it was to obtain information regarding them that these investigations were undertaken.

Thus far, important losses from chlorotic streak in Louisiana have been limited to relatively small areas where the virus became established before control measures were undertaken. Nevertheless, it is apparent from the losses already incurred, and from experimental results showing the severe reductions in germination of seed cuttings and ratoons that may occur (2, 3, 4), that the disease is of considerable potential importance. The fact that it can be held in check in moderately susceptible varieties by roguing seed plots (1, 2, 3), or eliminated from seed-cane cuttings by hot-water treatment (5, 8, 9, 11), lessens somewhat its threat to sugar-cane culture in areas where it has become established.

LOSS OF FOLIAGE SYMPTOMS

The loss of foliage symptoms from chlorotic-streak-infected sugar-cane plants has been noted previously (3, 10, 11). In the present study, this resulted principally from the death by senescence of the leaves on which the streaks occurred. Foliage symptoms were lost sometimes when normal green color was restored to indefinite, faintly chlorotic areas on infected leaves, but well-defined streaks did not disappear from living leaves.

During 1940 and 1941, loss of foliage symptoms in 4 varieties of sugar cane, the C.P. numbers 807, 28/19, 29/103, and 29/320, and its relation to the occurrence of the virus in the lateral buds of affected stalks, was studied

1 Pathologist, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture. The author is indebted to R. L. Tippett for assistance in some of the field and greenhouse experiments. in the field. Young plants obtained from diseased seed cuttings of each variety, and showing definite leaf streaks, were marked with numbered stakes in June. At intervals of approximately 15 days during the remainder of the growing season, leaf symptoms on the primary and secondary shoots were recorded. Each shoot in the stool was labeled with a numbered metal tag, the primary shoot being designated as number 1, and the tillers, or secondary shoots, numbered consecutively as they arose. In 1940, 100 stools each of C.P. 28/19 and C.P. 29/320 were studied, and in 1941, 100 of C.P. 29/320, 25 of C.P. 807, and 16 of C.P. 29/103.

The percentage of individual shoots with either temporary or permanent loss of foliage symptoms at some time during the season, and of secondary shoots remaining permanently free of such symptoms, are shown in table 1.

TABLE 1.—Percentage of primary and secondary shoots of 4 varieties of sugar cane showing temporary or permanent loss, or absence of foliage symptoms of chlorotic streak

				\mathbf{L}_0	ss of s	ymptom	s					
			Temp	orary				Perm	anent		Secondary	
C.P. variety					Sho	ots					shootspermanentlywithout	
	Pri-		Secon	dary		Pri-		Seco	ndary		symptoms	
	mary	1	2	3	4	mary	1	2	3	4		
807	20.0		6.0			32.0	18.0	12.5	33.3		44.4	
28/19	16.5	12.3	1.8	********	********	8.8	13.7	5.4	2.9	*******	11.7	
29/103	6.7	*******	20.0	7.7	*******	6.7	********	7.7		******	8.7	
29/320a	4.3	2.2	1.2		1.6	7.5		5.9	2.7	7.9	8.5	
29/320ь	16.8	3.3	********	moin		6.3	1.0	2.3	*********	*********	7.7	

a 1940.

Some plants of each variety had symptoms initially but became symptomless at some time during their growth. For the most part this temporary loss of symptoms occurred during the period of rapid growth in July and August, when the initially streaked leaves were lost through senescence and the new foliage produced was symptomless. During this time the new foliage also lacked the abnormal straightness and stiffness that characterizes many young plants in the field earlier in the season.

There was a tendency for primary shoots to lose symptoms more often than secondary shoots, although there were exceptions; and in C.P. 29/103 more of the secondary shoots recovered. Generally, loss of symptoms in primary shoots was by senescence of the affected leaves, whereas secondary shoots did not reach senescence during the period of observation.

Secondary shoots arising in diseased stools of C.P. 807 are much less likely to be invaded than are those of the other 3 varieties. All of the symptomless secondary shoots of C.P. 807 produced virus-free plants when indexed in the greenhouse, whereas diseased plants were produced from some of the symptomless secondary shoots of the other varieties. Recovery from

ь 1941.

foliage symptoms in infected shoots was more often permanent in C.P. 807 than in the other varieties.

DISTRIBUTION OF CHLOROTIC STREAK IN THE LATERAL BUDS OF INFECTED STALKS

According to Abbott (1), Wilbrink (11), and Bell (6, 7), not all of the lateral buds on chlorotic-streak-infected stalks of sugar cane give rise to diseased shoots when planted. However, the author's previous observations in this respect, and presumably those of Wilbrink and Bell, were on plants growing in the field where secondary spread of the virus was probably occurring, thus making impossible an exact determination of the extent of so-called recovery from the virus, and the distribution of infected buds on the stalk. The object of the present study was to obtain this information in an insect-proof greenhouse, and under conditions eliminating the possibility of secondary spread. Four commercial varieties of sugar cane grown in Louisiana, C.P. 807, 28/19, 29/103 and 29/320, were used, all of which have been observed to produce apparently healthy plants from diseased seed cuttings in the field.

Experiment 12. Two varieties, C.P. 28/19 and 29/320, were included in experiment 12, which was conducted during 1940–41. The final readings were made in late October and the symptoms estimated as mild if the leaf streaks were few or small and as severe if they were numerous, large, or necrotic. The stalks that had produced at least 5 mature lateral buds were harvested and taken to the greenhouse for indexing. There they were cut into single-bud cuttings, and the buds on each stalk numbered consecutively from the base upward. Missing and damaged buds were included in the seriation. The cuttings were potted in steamed, sandy-loam soil in 4-inch clay pots that had been immersed for 2 hours in a 1–250 solution of formaldehyde and placed on greenhouse benches where the air temperature was maintained at a minimum of 75° F.

Experiment 23. In experiment 23, which was conducted during 1941–42, 100 stools of C.P. 29/320, 25 of C.P. 807, and 16 of C.P. 29/103 were marked and observed as described under experiment 12. On 75 plants of C.P. 29/320, a record was kept of the total number of living leaves and the number with streaks at each observation date, with the view of obtaining a more accurate measure of symptom severity than the estimate made in experiment 12. In the fall the stalks were harvested and indexed. The buds of C.P. 29/320 were potted on September 22, 1941, and the plants not showing the disease were discarded on March 10, 1942. Those of C.P. 807 and 29/103 were potted on October 29 and 30, 1941, and discarded on July 3, 1942. All had been ratooned once.

It is possible that in these experiments additional plants would have eventually developed symptoms of chlorotic streak if they could have been held for longer periods, although it is improbable that the number would have been such as to affect the results materially. This was indicated by the fact that 10 plants of C.P. 29/320 from each experiment that were retained until they had produced jointed stalks, remained symptomless, as did the shoots produced when the lateral buds from these stalks were planted.

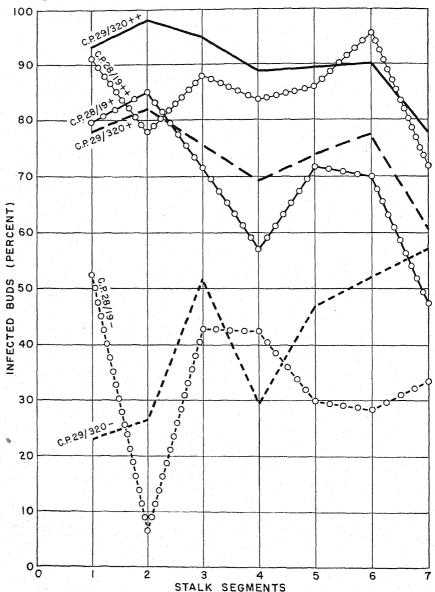


Fig. 1. Percentage of infected buds in segments of stalks of C.P. 28/19 and 29/320, classified according to severity of symptoms (Experiment 12): -, symptomless plants; +, mild symptoms; ++, severe symptoms.

For analysis of the results with respect to distribution of the virus in the lateral buds, each stalk was divided into 7 segments, each of which consti-

tuted approximately 14.3 per cent of the entire stalk. The object of such a division on a percentage basis was to place buds on different stalks of pre-

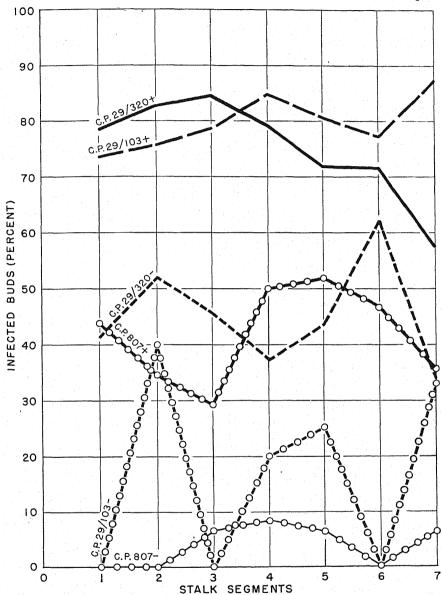


Fig. 2. Percentage of infected buds in segments of stalks of C.P. 807, 29/103, and 29/320, classified according to presence or absence of symptoms (Experiment 23): ~, symptomless plants; +, plants with symptoms.

sumably similar physiological and anatomical development in the same relative groups. For example, bud number 10 on a long stalk of 15 to 18 buds might differ physiologically from bud number 10 on a shorter stalk on which

it was the top bud. The results of the experiments are presented graphically in figures 1 and 2.

In experiment 12 (Fig. 1) there was a tendency for greater freedom from the virus in the upper portions of the stalks that had leaf symptoms. Otherwise there was no consistent tendency for the virus to be localized in any portion of the stalk. In both experiments, distribution in the symptomless plants was more erratic than in those with symptoms. There was a definite relationship between severity of symptoms and the number of infected buds. The symptomless stalks produced the lowest number of diseased plants, those with severe symptoms the highest, while those with mild symptoms were intermediate.

These relationships are emphasized by the frequency distribution in table 2, in which the data are arranged according to the percentage of buds

TABLE 2.—Distribution of stalks of 2 varieties of sugar cane in frequency classes of percentages of buds per stalk producing diseased plants. Experiment 12, stalks indexed in the greenhouse

Variety and symptom	Distribution of stalks in frequency classesa									
severity in field when harvested	0	1-20	21-40	41-60	61-80	81–99	100			
and the second s	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct			
C.P. 28/19										
Symptomless	41	6	6	12	18	12	6			
Mild		8	8	15	35	19	15			
Severe		******		9	16	28	47			
C.P. 39/320										
Symptomless	19	6	31	6	25	6	6			
Mild	2	*****	9	9	18	39	23			
Severe				3	5	49	44			

^a Classes based on percentage of buds per stalk producing diseased plants.

per stalk producing diseased plants. With both varieties, nearly half of all the stalks in the group with severe symptoms produced diseased plants from all of their buds. In the symptomless group, 41 per cent of the stalks of C.P. 28/19 produced all healthy plants, whereas only 19 per cent of those of C.P. 29/320 were healthy.

That severity of foliage symptoms at harvest time was related to virus invasion of the buds was further indicated by the record made of the percentage of streaked leaves on 75 plants throughout the season in experiment 23. When the results obtained were arranged in frequency classes according to the percentage of leaves with streaks at the time of harvest, it was found that on stalks with 1 to 20 per cent of their leaves streaked, 61 per cent of the buds produced diseased shoots; in the groups with 21 to 40 per cent, and 41 to 60 per cent of the leaves streaked, 81 per cent of the buds in each were diseased; and in the group with 61 to 80 per cent of the leaves streaked, 95 per cent of the buds were diseased.

Nevertheless, the occurrence of streaks on a leaf did not necessarily indicate infection of the bud on the node subtending it. Some stalks with dis-

seased leaves produced only apparently healthy plants when their lateral buds were germinated. Symptoms on individual leaves of 6 plants of C.P. 29/320 in the greenhouse were recorded from germination until plants were about 6 months old and had produced 7–10 lateral buds of sufficient maturity to germinate. From buds subtending diseased leaves 19 diseased and 13 healthy plants were produced; and from buds subtending healthy leaves 2 diseased and 4 healthy plants were produced.

There was no consistent relationship between the percentage of germination of buds on stalks in the different symptom groups and the occurrence of chlorotic streak. Because of the known adverse effect of the virus on germination in the field, it might have been expected that stalks or segments with the lowest incidence of infection would have the highest germination. This, however, was not true, the average germination being approximately the same regardless of symptoms. This may have been at least partially the

TABLE 3.—Recovery from chlorotic streak in foliage and buds of entire stools of 4 varieties of sugar cane

		Stools showing complete recovery in:				
C.P. variety	Expt. No.	Total No. of stools	Folia	Buds		
		Stoois	Temporarya	Permanent	Buncr	
			Pct.	Pct.	Pct.	
807	23	25	33.3	25.0	16.7	
28/19	12	100	17.0	5.0	2.0	
29/103	23	16	0.0	0.0	0.0	
29/320	12	100	7.0	5.0	2.0	
29/320	23	100	6.0	1.0	4.0	

^a Foliage of all shoots of the stool symptomless at some time, developing symptoms again later in the season.

result of the favorable temperature for rapid germination of sugar cane in the greenhouse, at which the virus had no marked influence on germination.

Recovery in the Buds. The results were examined for evidence of recovery from the disease in the buds, aside from the loss of foliage symptoms, which was discussed earlier. Some of the data obtained may be interpreted as evidence of such recovery. In table 3, data from experiments 12 and 23 are presented on the basis of entire stools, rather than for individual shoots (as in table 1); and in table 4, the records of symptoms in the field and the number of lateral buds producing diseased plants in the greenhouse are summarized for 9 typical stools.

Some of the stools of C.P. 807, 28/19, and 29/320 showed complete loss of foliage symptoms in all of their shoots, and in some of these the virus was apparently not present in the lateral buds at harvest time, as evidenced by the absence of symptoms in the plants grown from them (Table 4). Examples are C.P. 807, stool 7, C.P. 28/19, stool 20, and C.P. 29/320, stool 56 (Table 4). This is evidence of recovery if it can be assumed that the buds actually had been invaded by the virus. That such an assumption is justi-

fied is indicated by the fact that in experiments 12 and 23, 83 per cent of the stalks of C.P. 807 with foliage symptoms at some time during the season were found to have some infected buds, 93 per cent of those of C.P. 29/103, 95 per cent of those of C.P. 29/320, and 99 per cent of those of C. P. 28/19.

TABLE 4.—Occurrence of chlorotic streak in leaves and buds of individual stalks of $\mathcal S$ varieties of sugar cane

a D	Stool	Leaf symptoms ^c						Buds	Buds indexed	
C.P. varietya	and shoot No.b	May	June	July	Aug.	Sept.	Oct.	Total	No. with C.S.	
807	7-1		+	+	+	_	_	12	0	
	7-2	*****		-	_		_	12	0.	
	7-3			*****	-		· · ·	10	0	
	11-1	4	+	+		_	+	16	0	
	11-2	******	-	_	+			10	0	
	11-3	******		+ 1	+	+	1 +	4	4	
	11-4		******	·	+	_	-	8	0	
	17 - 1		+	+	+	+	+	15	6	
	17-2		******		+	_	-	6	0	
28/19	20-1	*****			_			1.1	0	
	20-2		+				******	9	0	
	20-3	******	+	+	+	_	******	9	. 0	
29/320	6-1	+	+			-		6	6	
	6-2	+	+	4.	+	+		4	4	
	56-1	+	+	+				$1\overline{2}$	ō	
	56-2			-		_	*****	8	ŏ	
	56-3	*****	*****	_			*****	5	0	
	57-1	+	+	+ .	+	+	******	13	. 11	
	57-2		-	•	-	-	******	11	11	
	57-3		-		-		*****	8	. 8	
	57-4		_				******	4	3	
	82-1	+	+.*	-	+	+	*****	9	3	
	82-2	******	-				*****	6	0	
	82-3	*****	, - ',	-		-	*****	8	0	
	82-4	m	******	******	_		*****	9	0	
	96-1	+	+	+		-	,,,,,,	9	. 0	
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	99-2	+	+	+	+		*****	8	0	
	99-2 99-3	*****	- -	·	-	-		9	2	
	99-4		-	-	+ .	+		8	6	
	00-t	*****	*****		-	+	*****	4	3	

^a Material of C.P. 28/19 was from experiment 12; all other material was from experiment 23.

b The primary shoot in each stool is no. 1; the secondary shoots, 2 to 4, according to the order in which they arose in the stool.

c+indicates symptoms present; -indicates symptoms absent.

That the loss of foliage symptoms was associated with a decrease in incidence of the virus in the buds is further shown by table 5, in which data from experiments 12 and 23 are presented on the basis of the occurrence of chlorotic streak in the buds in relation to the constancy of leaf symptoms during the period from May to October. On the whole, the buds of plants showing leaf symptoms constantly throughout the season were invaded to a greater extent than those showing foliage recovery at some time. There were excep-

tions to the general rule, however, for some individual plants that had leaf symptoms at only the final reading produced diseased plants from all of their lateral buds, and others that were symptomless for as long as 3 months produced infected plants from a majority of their buds (Table 5). The groups of stalks of C.P. 29/320 that were symptomless constantly in experiments 12 and 23 (all secondary shoots) also showed a higher percentage of diseased buds than did those that had symptoms at some time, but had recovered by harvest. While the number of stalks studied in these groups was too small for the results to be considered conclusive, these data, together with those for the symptomless stalks, indicate that stalks of C.P. 29/320 are more likely to be infected with the chlorotic-streak virus without showing leaf symptoms, than are those of the other 3 varieties studied.

TABLE 5.—Occurrence of chlorotic streak in lateral buds of 4 varieties of sugar cane in relation to the constancy of leaf symptoms²

		Occurrence of leaf symptoms during the season ^b									
C.P. variety	Expt. No.			Alternating + and - but + at harvest		Alternating + and - but - at harvest		continuously			
		Stalks	Buds C.S.	Stalks	Buds C.S.	Stalks	Buds C.S.	Stalks	Buds C.S.		
807 28/19 29/103 29/320 29/320	23 12 23 12 23	No. 21 90 39 79 70	Pct. 49.8 77.1 83.1 81.5 77.0	No. 5 6 4 4 15	Pct. 34.8 45.5 55.1 78.0 63.4	No. 17 12 2 12 9	Pct. 15.2 49.5 40.0 33.6 41.9	No. 11 6 2 4 13	Pct. 3.9 7.8 0 70.6 45.0		

^a Includes only those stalks observed for at least 3 months. ^b + indicates symptoms present; – indicates symptoms absent.

It is possible that in the recovered plants the virus had been present only in leaf and stalk tissues, and had not invaded the lateral buds. It will be noted in table 4, for example, that several stalks with leaf symptoms in the field produced apparently healthy plants when their buds were planted in the greenhouse. Later, when buds on stalks grown from these plants were germinated, only apparently healthy plants were produced. It is possible that the buds on the original plants were never invaded by the virus.

In the absence of some means of definitely determining the presence of the virus in the plant other than the occurrence of the leaf streaks, it is difficult to obtain positive proof of recovery. Considering all of the evidence, however, it seems reasonable to assume that at least some of the lateral buds on the stalks that apparently recovered had been invaded by the virus, and that the absence of the disease in the plants produced from them is indicative of recovery during or preceding germination.

That the stalk and not the stool is the physiological unit involved in the distribution of the virus in the plant, or in recovery from the disease, is evi-

dent from the data in table 4. The disease appeared in or disappeared from individual stalks of a stool irrespective of other stalks comprising the stool.

MOVEMENT OF THE VIRUS WITHIN THE STOOL

Natural Movement. The field studies indicated that chlorotic-streak virus spreads from infected to healthy shoots comprising a stool of sugar cane (Table 4). However, since the field-grown plants were exposed to secondary spread by insects, it was uncertain whether the appearance of the disease in previously healthy shoots of a diseased stool resulted from transfer by insects, or from movement into that shoot from another diseased shoot. Proof of such movement has been obtained with plants grown from diseased single-bud cuttings in steamed soil in an insect-proof greenhouse. The plants were started in 4-inch pots and later transferred to 4-gallon cans, where they remained until several tillers had produced jointed stalks with mature buds. The results with several plants of the variety C.P. 29/320 are presented in table 6.

TABLE 6.—Occurrence of chlorotic streak leaf symptoms in secondary shoots arising from diseased primary shoots of the sugar-cane variety C.P. 29/320

		Sympt			
Plant No.			Secondary shoots		
	Primary shoot—	1	2 3	4	
12-2-1 12-2-9 40-1-1 40-1-10 40-3-10 85-3-6 II-D-93	+ + + + + + + + + + + + + + + + + + + +	+ + + - - - + +	 + + 		

^{*+}indicates symptoms present; -indicates symptoms absent.

The virus may spread to all of the secondary shoots (Plant No. 85–3–6), to none of them (Plant No. 40–3–10), or to some and not to others. That the symptomless secondary shoots were not invaded was indicated when the mature lateral buds produced on them were planted in the greenhouse, and the resulting plants remained free of chlorotic streak symptoms. It is possible that the apparently symptomless shoots were invaded, but that the concentration of virus was insufficient to induce the characteristic leaf symptoms.

Effect of Severing the Primary Shoot. Removing the primary shoot from a stool of sugar cane usually stimulates the development of new secondary shoots and the growth of those already present. Presumably food materials stored in the underground portion of the stem of the primary shoot move into the secondary shoots in this process. The percentage of plants with chlorotic-streak symptoms is generally greater in fields of rations than in the same fields when they were in plant cane, and it is possible that the appear-

ance of the disease in the shoots arising following ratooning may be correlated with movement of reserve food materials into them.

To determine the effect of severing the infected primary shoot on the development of chlorotic streak in secondary shoots previously showing no indication of the disease, 6-month-old plants of the variety C.P. 29/320 growing in 4-gallon cans in the greenhouse were selected, all of which showed chlorotic streak symptoms in the primary shoot, but none in any of the secondary shoots. There were from 1 to 6 secondary shoots per plant and each shoot had from 4 to 10 unfolded leaves. The primary shoot was cut from some of the plants immediately above the point of origin of the first secondary shoot, and the others were left as controls. At intervals thereafter notes were made on the occurrence of chlorotic streak in the secondary shoots. The results of 2 typical experiments are presented in table 7.

TABLE 7.—Effect of severing the primary shoot on the appearance of chlorotic streak symptoms in the secondary shoots of stools of the variety C.P. 29/320

T)			Secondary shoots						
Duration of expt.	Treatment	Number of stools		eginning expt.	At end of expt.				
Days			Total No.	With C.S.	Total	With C.S.			
				No.	No.	No.	Pct.		
90	Control 1 shoot severed	10 10	16 28	0	54 57	6 32	11.1 56.1		
30	Control 1 shoot severed	6 8	15 18	0	15 21	$\begin{matrix} 6\\16\end{matrix}$	$\frac{40.0}{76.2}$		

It is apparent that removing the infected primary shoot stimulated the movement of the virus into the secondary shoots. Approximately twice as many in one experiment, and 5 times as many in the other, developed symptoms of the disease in the treated as in the untreated plants. Similar results were obtained when the primary shoot was removed from plants grown from infected seed cuttings, but in which all shoots had remained symptomless up to the time the primary shoot was severed.

Since reserve food materials presumably moved from the underground portion of the stem into the secondary shoots following the cutting of the primary shoot, it may be assumed that the appearance of chlorotic streak in the secondary shoots was correlated with this movement. Such an assumption offers a plausible explanation for the commonly observed higher incidence of chlorotic streak in ratoons as compared with plant cane in the field. Presumably this results from the development of symptoms in plants that are infected as plant cane, but in which foliage symptoms do not appear until they are ratooned. It is recognized that the plants in these experiments, in which infection was primary, i.e., from the seed cutting, were not strictly comparable with field-grown plants which become infected through secondary spread by insect vectors. However, in both instances, the physiological

process of ratooning and the consequent movement of the virus into the new shoots would be analogous.

DISCUSSION

The studies reported in this paper explain the often-observed transient nature of chlorotic streak in the field, showing that the apparent decline in incidence of the disease during the season of rapid sugar-cane growth in summer results from loss of foliage symptoms through the senescence of streaked leaves and the absence of streaks in the new foliage. In general, this was associated with a reduction in the number of infected buds, but complete recovery in both foliage and buds apparently occurred in a relatively small percentage of the stools studied. The majority of the plants were only transiently symptomless.

From a practical standpoint, the significance of the results lies in showing the desirability of roguing seed-cane plots for chlorotic streak early in the season, i.e., in May and June, before the rapid summer growth begins, and before many infected plants lose external symptoms of the disease. Although the percentage of completely symptomless infected stools at any one time during the season was not high for C.P. 29/320, it was considerable for C.P. 28/19 and C.P. 807 (Table 3). Considering also the greater difficulty of detecting the often inconspicuous symptoms in the larger cane during the summer, as well as the greater expense resulting from the increased time required for inspecting the taller cane, the importance of early roguing is further apparent. Practical experience has shown that if the diseased stools are eliminated in May and June, infection may be reduced to a very low point without summer roguing (1, 2, 3).

The sugar-cane varieties studied differed in the extent to which shoots and buds of infected plants were diseased. This may have been the result of more restricted movement of the virus in the plants of some varieties, a greater degree of recovery, or both. These differences in relative degree of distribution of the virus were not correlated with relative susceptibility to infection. C.P. 807, for example, which is about as susceptible to infection as C.P. 28/19 (4), recovered from foliage symptoms to a considerably greater degree than the latter variety, and the virus was present in a lower percentage of the secondary shoots and lateral buds. Likewise, C.P. 29/320, which is near C.P. 29/103 in susceptibility to infection (4), showed a greater degree of foliage recovery and a lower percentage of infected lateral buds. Varieties of equal susceptibility to infection also differ in the extent to which their germination and yields are reduced by the disease (4). It is apparent, therefore, that a true appraisal of the importance of the disease in a variety must take into account its ability to recover from and to resist the deleterious effects of the virus, as well as its susceptibility to infection.

The possibility that sugar-cane plants may harbor the virus of chlorotic streak without showing the characteristic leaf streaks, introduces an element of uncertainty into experimental work, the results of which depend on deter-

mining the presence or absence of the virus in a plant. Admittedly, some of the results reported in this paper may have been influenced to some extent by this uncertainty. It was found, however, that in the greenhouse, leaf symptoms could be induced in many plants that were previously symptomless, by a heavy application of NaNO₃. Plants that failed to develop symptoms following this treatment did not subsequently show any indication of the presence of chlorotic streak, even though some of them or their progeny were carried for 2 years in the greenhouse. It is believed, therefore, that the number of plants in these experiments that may have harbored the virus without showing symptoms is very small, and that the results were not affected materially thereby.

SUMMARY

Chlorotic-streak-infected sugar-cane plants became symptomless through the loss by senescence of the streaked leaves, and the temporary or permanent failure of symptoms to develop in the foliage subsequently produced. Less frequently, foliage recovery occurred through the restoration of the normal green color in faintly chlorotic areas of diseased leaves, but disappearance of well-defined streaks from affected tissues was not observed.

On the whole, the extent of infection of the buds was correlated with the severity of foliage symptoms, and was greater in those plants that had symptoms continuously than in those that showed temporary or permanent loss of symptoms. However, loss of foliage symptoms was not necessarily associated with disappearance of the virus from the stalks: in some plants the presence of the virus in the buds was demonstrated several months after the leaf symptoms disappeared.

Some buds from infected stalks produced apparently healthy plants on germination. It is assumed that this was the result of actual recovery from the disease, although it is possible that these buds had not been invaded by the virus, or that the concentration of virus was insufficient to induce the characteristic leaf symptoms. There was a tendency for the buds on the upper segments of the stalks to have greater freedom from the disease than the lower.

The sugar-cane varieties studied differed in the extent to which shoots and buds of infected plants were diseased. This may have been the result of a more restricted movement of the virus in the plants of some varieties, a greater degree of recovery, or both.

The stalk rather than the stool of sugar cane was found to be the physiological unit involved in the distribution and movement of the virus in the plant. The disease appeared in or disappeared from individual stalks independently of the rest of the stool.

Removing the infected primary shoot from a stool of cane with initially healthy secondary shoots caused a marked increase in the number of diseased secondary shoots. It is postulated that the appearance of the disease in the secondary shoots may have been correlated with the movement of reserve materials into them from the underground portion of the stem of the infected

primary shoot. This offers a plausible explanation for the greater incidence of the disease in rations as compared with plant cane.

DIVISION OF SUGAR PLANT INVESTIGATIONS,

U. S. DEPARTMENT OF AGRICULTURE.

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PHYTOPATHOLOGICAL NOTES

Virus Diseases of Guayule.—The reactions of guayule seedlings to a number of plant viruses have been tested experimentally.

Ordinary tobacco-mosaic virus. Guayule seedlings were experimentally infected with ordinary tobacco-mosaic and tobacco-ring-spot viruses by mechanical inoculation using the carborundum method. Seedlings inoculated with the tobacco-mosaic virus developed small necrotic areas on the inoculated leaves but not on those newly developing. The virus was recovered and transferred to Nicotiana glutinosa from the inoculated leaves but not from the newly developing leaves. Local lesions appeared on the inoculated leaves of N. glutinosa. The infection of guayule seedlings was local and not systemic.

Tobacco-ring-spot virus. Several hundred guayule seedlings inoculated with the tobacco-ring-spot virus were symptomless carriers of the disease. The virus was recovered from the inoculated leaves and transferred to Nicotiana glutinosa. The infection was local and not systemic.

Cucumber-mosaic viruses. Fifty-five potted guayule seedlings were non-susceptible to western cucumber-mosaic virus, and 30 seedlings were non-susceptible to the ordinary cucumber-mosaic virus. In flats containing approximately 100 plants, no infection was observed.

Celery-mosaic viruses. There was no infection in 50 potted guayule seedlings inoculated with virus of western-celery-mosaic, nor in 25 seedlings inoculated with celery-calico virus. Plants growing in flats also were non-susceptible.

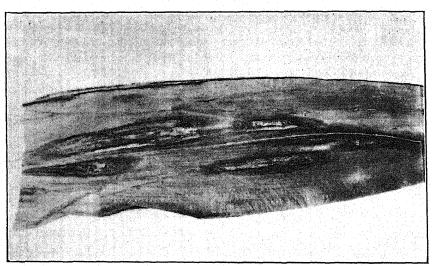
Beet-mosaic virus. Twenty-five potted guayule seedlings were non-susceptible to the beet-mosaic virus, as were approximately 100 plants grown in flats and inoculated with this virus.

Sugar-beet curly-top virus. Lots of 20 infective beet leafhoppers, Eutettix tenellus (Baker), were used to inoculate guayule seedlings with the curly-top virus but no symptoms of the disease developed. Noninfective beet leafhoppers failed to recover the virus and transfer it to healthy sugar beets. Nymphs which hatched from eggs deposited in guayule seedlings died.

California-aster-yellows virus. Attempts were made to infect guayule seedlings with the California-aster-yellows virus. The following species of leafhoppers, vectors of this virus, were used to inoculate the plants and non-infective adults of each species failed to recover the virus and transfer it to healthy aster and celery plants: short-winged aster leafhopper, Macrosteles divisus (Uhl.); long-winged aster leafhopper, a race of the same species, M. divisus; mountain leafhopper, Colladonus montanus (V. D.); geminate leafhopper, Idiodonus geminatus (V. D.); Phlepsius lathropi Baker, P. latipex De L., P. spatulatus V. D., Acinopterus angulatus Law., and Cloanthanus irroratus (V. D.).

Toxic salivary secretion. The toxic salivary secretion of Xerophloea vanduzeei De L. induces some of the symptoms closely resembling curly top on sugar beets and aster yellows but this leafhopper failed to produce symptoms on guayule seedlings.—Henry H. P. Severin, Guayule Research Project, Bureau of Plant Industry, United States Department of Agriculture and College of Agriculture, University of California.

The Sooty Stripe Disease of Sorghum.—The fungus, Titaeospora andropogonis (Miura) Tai, described as the cause of a serious leaf disease of Kaoliang sorghum in China and Manchuria, was first reported in the United States in 1942.2 Since then the fungus has apparently been spreading in Louisiana and Mississippi and at this time it seems advisable to present the information which is now available concerning the disease it causes.



Lesions caused by Titaeospora andropogonis on leaf of sorghum. size.

In many respects the disease (Fig. 1) resembles the leaf blight caused by Helminthosporium turcicum Pass. The lesions are elongate elliptic, up to several centimeters long and 1-2 centimeters wide, rather regular in outline, light brown to grayish in the central portion, and bordered by a narrow to broad deep red margin. On the lesions, numerous, small, rough, spherical or subspherical black bodies are usually present, though these are easily brushed off. The occurrence of the black bodies readily distinguishes the disease from leaf blight, and it is due to their presence that the name sooty stripe is suggested. In Manchuria the black bodies appear in late autumn, 1 but in the United States they have been observed as early as July.

¹ Miura, M. Diseases of principal crops in Manchuria. Koshurci Agr. Exp. Sta.

of the S. Manchurian Railway Co., Report No. 11. Jan., 1921.

² Bain, D. C., and C. W. Edgerton. Two leaf-spot diseases on sorghum and related grasses. Phytopath. 32: 1. 1942.

Thus far, the disease has been reported only in China, Manchuria, and the United States. In Louisiana and Mississippi, it has been found on several varieties of sorghum and on Johnson grass (*Holcus halepensis*).

Miura described the fungus in 1921 and placed it in a new genus, Ramulispora. In 1932, however, Tai3 redescribed it and placed it in the genus Titaeospora described by Bubak⁴ in 1916. Miura's description of the fungus is as follows: "Conidiophores hyaline, non-septate, slender, 20-35 $\times 2.5~\mu$, springing from subepidermal black stroma. Conidiospores cylindrical, flexuose, slender, branched, 4-12 septate, not restricted at septum, 36-100 × 2-4 µ, granular, hyaline. Chamydospores intercalary or terminal, usually several in a chain, yellowish brown, globose or ovate, about 15 μ in diameter, with a few small spinules." The black bodies were referred to as "black bodies of unknown nature." Studies in Louisiana indicate that these black bodies are sclerotia and are possibly formed after sporulation. While Miura placed the fungus in the Melanconiales, recent studies indicate that the fruiting structure is not a typical acervulus but rather a sporodochium which arises immediately below the stomate. Whether Miura's "subepidermal black stroma" is formed before or after sporulation is a matter yet to be determined.

In culture the fungus grows slowly and the mycelium forms a rather dark greenish-gray clump. Conidia are produced in abundance in pinkish thread-like masses in 3-4 days after transferring. Conidia germinate readily within 20 hours. No sclerotia have been observed in culture. Chlamydospores develop readily in bean-pod agar.

Johnson grass and several varieties of sorghum in the greenhouse and field were inoculated in the spring and summer of 1942. Infection was obtained, usually 8–10 days following inoculation, but symptoms as described previously did not appear by September. The fungus was recovered from the small, round to elliptic spots which resulted from the inoculations. The spots attained their maximum size on the sorghum varieties, White Kaoliang, C.P. Special, Standard Broomcorn, and Dwarf Yellow Milo.—Douglas C. Bain, Department of Botany, Louisiana State University, Baton Rouge, La.

Rhizoctonia Foliage Disease of Hevea brasiliensis.—During a study of Rhizoctonias affecting sugar-beet foliage opportunity was afforded to compare these organisms with a fungus affecting leaves of Hevea brasiliensis (H.B.K.) Muell. Arg. Specimens of the affected leaves had been referred to this Bureau by Rolland C. Lorenz, of the Agricultural Experiment Station at Tingo Maria, Peru. The Hevea leaves examined had spots varying from 1 to 10 mm. or more in diameter to blighted areas involving one-half to two-thirds of the leaf blade. The affected areas were commonly surrounded by a narrow, brown border. Within the border a portion of the affected area was white, attributable to the destruction of the parenchyma, the trans-

<sup>Tai, F. L. Notes on Chinese fungi. I. Nanking Journ. 2: 171-179. 1932.
Bubak, F. Pilze von verschiedene Standorten. Ann. Mycol. 14: 341-352. 1916.</sup>

parent cuticle being left. The white portions of the spots apparently resulted from primary invasions. Successive advances of the fungus from the earlier invaded tissues occurred and were evidenced by collapsed tissue, usually light brown, surrounding the older spots. Brown-tinged, Rhizoctonia-like mycelium was found on the surface of the leaves.

Microscopic examination of the fungus found on the leaves collected at Tingo Maria, Peru, showed it to be of Rhizoctonia type. Distinct rhizomorphs, as reported for *Pellicularia koleroga* (Cke.) Rogers on various hosts, were not present. The hyphae measured 6.5 μ in diameter. The perfect stage of the fungus occurred as grayish white, powdery areas on both sides of the affected leaves. Judging by their abundance, the basidiospores could be a major factor in dissemination.

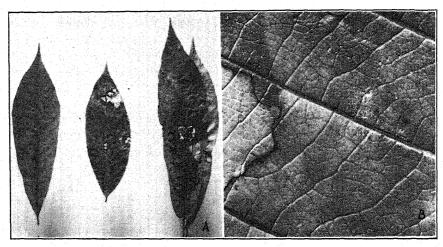


Fig. 1. Rhizoctonia foliage disease of Hevea brasiliensis. A. Comparison of a healthy Hevea leaf (left) with infected leaves. The leaf in the center is a naturally infected leaf collected at Tingo Maria, Peru. The leaves at the right were artificially infected with a pure culture of the fungus (R 435) isolated from the material from Peru $(0.25\times)$. B. Perfect stage of the organism as produced from an artificial inoculation with the pure culture. In naturally occurring infections, larger areas on both dorsal and ventral surfaces of the leaf may be covered by the spore-producing stage of the fungus $(2.5\times)$.

The causal fungus was isolated from a diseased leaf and its pathogenicity tested on greenhouse-grown, rubber-plant seedlings. From a resulting leaf spot the fungus was reisolated and used to inoculate young and old leaves of rubber plants as before. At 21° to 25° C. and with relative humidity at 90 to 100 per cent, infection of young leaves occurred in 5 days (Fig. 1, A) and the perfect stage developed 4 or 5 days later (Fig. 1, B). Leaf penetration was effected from minute infection cushions. No rhizomorphs were found.

On sugar beets under similar environmental conditions foliage infection and formation of the perfect stage of the fungus occurred in 10 to 15 days. The fungus did not cause damping off of sugar-beet seedlings or the rotting of mature roots.

The perfect stage arises from the vegetative mycelium as closely intertwined, more or less dichotomously branched, hyphae tightly adherent to the leaf surface. The basidia are terminal and bear four sterigmata and spores. The sterigmata are approximately the same length as the longest diameter of the basidiospores. Fifty spores obtained from one of the infected leaves of a rubber plant were measured and averaged $8.2\times3.7\,\mu.$ This spore size and other characters of the fungus conform with Pellicularia filamentosa (Pat.) Rogers, as recently proposed. It is to be noted, however, that the perfect stage of the Rhizoctonia affecting Hevea foliage is composed of tightly intertwined hymenial cells and is strongly adherent to the surface of the leaf on which it is formed. It is not readily removable. This is in contrast to the loose, rather filamentous structure of the hymenial stage of many other representatives of this admittedly composite species.

The mycelial mat of the fungus when grown on potato-dextrose-agar medium, is brownish black² (5 YR $0.7/0.8^{a}$) and the substrate is heavily darkened as result of the growth of the fungus. The sclerotia are loose textured and, rather uniformly, about 2 mm. in diameter. The perfect stage of the fungus was not observed to occur on this medium.—John E. Kotila, Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Md.

Cotton-Leaf-Spot Rhizoctonia and Its Perfect Stage on Sugar Bects.— In connection with determining the pathogenicity to sugar beets of several isolates of Rhizoctonia, an organism, isolated by D. C. Neal from a leaf of cotton affected with Rhizoctonia leaf spot, was included.1

Under conditions favorable for damping off of seedlings and rotting of older roots of sugar beets the cotton-leaf-spot Rhizoctonia was pathogenic on seedlings causing a damping off that started 3 or 4 days after emergence of the seedlings and in 10 days reduced the stand to 61.5 per cent of the control. The fungus was not pathogenic on older roots.

The cotton-leaf-spot Rhizoctonia was mildly pathogenic on the foliage of sugar-beet plants kept at 21° to 25° C. and a humidity of 90 to 100 per cent. Infection occurred in 4 days after inoculation but the infected areas remained small. Ten days after inoculation the largest area measured approximately 2×4 cm.

The mycelium of the fungus grew over the surface of the leaf from the sand-corn-meal inoculum as well as from the infected spots and produced the perfect stage of the fungus (Fig. 1), which according to Neal has not been observed on cotton. The hymenial stage appears as a grayish white

¹ Rogers, Donald P. The genus Pellicularia (Thelephoraceae). Farlowia 1: 96-118.

² Judd, Deane B., and Kenneth L. Kelly. Method of designating colors. Natl. Bur.

Standards Res. Paper Rp1239. 1939.

3 Munsell Book of Color. Revised Text, 1942. Munsell Color Company, Inc., Baltimore, Md.

Neal, D. C. Rhizoctonia leaf spot of cotton. Phytopath. 34: 599-602. 1944.

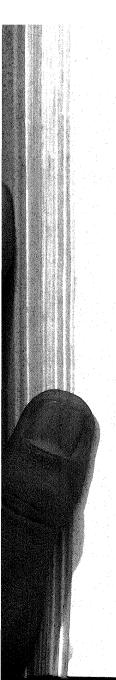
powdery growth, readily removable for microscopic examination when wetted with a fine spray of water.

The hymenial cells, basidia, sterigmata, and basidiospores are similiar



Fig. 1. Sugar-beet leaf affected by the cotton-leaf-spot *Rhizoctonia*. The dark, sunken spots are the invaded portions of the leaf. The raised, cottony masses on the leaf surface are vegetative mycelial growths from the inoculum. The perfect stage of the fungus was produced on the leaf surface and appears as a white, powdery growth. The white areas (a) at the top and center of the photograph show the more pronounced development of hymenial structures, but along the veins and rather generally on the leaf surface, other hymenial aggregates occur.

to Rhizoetonias formerly grouped as Corticium vagum B. & C. In 50 measurements, spore size averaged $8.8\times6.9\,\mu$. The sterigma length was approximately equivalent to the longest spore diameter.



Spore size and other characters place the fungus in the species *Pellicullaria filamentosa* (Pat.) Rogers² as recently proposed.—John E. Kotila, Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Md.

The Longevity of the Pathogen Causing the Wilt of the Common Bean.—In 1930 the writer pointed out that the pathogen Corynebacterium flaccumfaciens (Hedges) Dowson, the cause of the wilt of the common bean, is a bacterium very tolerant to the environment under which it is found and that it can remain viable in dried leaves in the herbarium for at least 5 years. Later Christow in Germany presented evidence which indicated that the pathogen could remain alive for 7 years in bean seed. The 7-year-old seed he used germinated and gave rise in a few cases to wilted plants typical of the disease.

Recent tests by the writer show that Corynebacterium flaccumfaciens will live for 24 years in association with bean seed under room conditions. In 1919 a handful of infected White Marrow bean seed was collected and stored in a screw-top glass container, labeled, and placed on a shelf in a laboratory at Cornell University. All these seed had definite yellow varnish-like lesions more or less typical of the wilt disease. After 15 years of storage, experiments were conducted to determine whether or not the pathogen in the seed was still viable and virulent. Dilution plates were made from 3 different seed and in each instance yellow bacteria were isolated that appeared in all respects to be C. flaccumfaciens. Inoculations on Red Kidney bean plants growing in the greenhouse proved that the bacteria were pathogenic. At the end of 20 years, in 1939, similar experiments were conducted with like results. In 1943, at the end of 24 years storage, three seed again were tested, but only one yielded viable and virulent cultures, and in this instance the colonies were not so numerous on the plates as they had been in the previous tests. The following year the remaining seed, 10 in all, were tested for living bacteria, but in no case were any organisms found that were able to grow. Twenty-four years appears to be the limit of the pathogen's viability under the conditions in which they were stored. The room was warm and dry, but not excessively so. The bacteria also were further protected underneath the seed coat where they had dried down in the plant juices and were not exposed to the changes, however slight, that went on in the tight glass container. In some respects the pathogen was treated and stored under conditions somewhat similar to those of the Swift method³ for desiccating and storing bacterial cultures.

² Rogers, Donald P. The genus Pellicularia (Thelephoraceae). Farlowia 1: 96-118. 1943.

¹ Burkholder, Walter H. The bacterial diseases of the bean: a comparative study. New York (Cornell) Agr. Exp. Sta. Mem. 127: 36. 1930.

² Christow, Alexander. Einige Versuche über die Bakterienkrankheiten bei Bohnen.

Phytopath. Ztschr. 7: 537-544. 1934.

³ Swift, H. F. A simple method for preserving bacterial cultures by freezing and drying. Jour. Bact. 33: 411-421. 1937.

Xanthomonas phaseoli (Smith) Dowson, on the other hand, is not supposed to remain viable in or on the bean seed for much over a year. Rapp⁴ states that this pathogen is dead in 2- and 3-year-old seed, and Christow² found them non-viable in 7-year-old seed. As far as the writer is aware, nothing has been published on the longevity of Pseudomonas phaseolicola (Burkholder) Dowson, the cause of halo-blight of the bean.—Walter H. Burkholder, Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Phoma terrestris on Sugar-Cane Roots in Louisiana.—Phoma terrestris has been isolated many times during the years 1942 to 1944 from the roots of a number of cane varieties secured from various localities in Louisiana. The same fungus has been isolated also from onion, garlic, sweet clover (Melilotus indica), and corn roots as well as from cane, rice, and cotton soils. All isolates were identical in their cultural and morphological characters. The identity of the fungus was verified by Dr. E. C. Tims. The pyenidial stage was readily obtained with all isolates on potato-dextrose agar at room temperatures in subdued daylight.

Inoculations of the cane-root isolates into corn roots were made as follows: yellow corn seeds were germinated under sterile conditions in moist chambers. When the corn roots were from a half to one inch in length, a small piece of agar bearing the actively growing fungus was placed at the tip of each. After inoculation, the plates (moist chambers) were incubated at room temperature and in the dark. After 7 to 15 days, the results were obtained. Cross and longitudinal sections (free-hand) of the affected portions of the inoculated roots were made. It was found that after one week the fungus had usually penetrated the cortex and in some instances the stele as well, although the cortex was the region more generally attacked. the isolates of Phoma terrestris gave similar results. The tissues affected by the organism turned red-brown. Very often the invaded host cells were entirely filled by the mycelium. The endodermis showed great resistance to the entrance of the fungus mycelium. The roots usually do not get flabby; some get stiff. Other isolates from onion, garlic, and corn, inoculated into corn roots, gave similar results.

These findings demonstrate that *Phoma terrestris* is widely distributed in the soils of the State of Louisiana and most of the isolates penetrated and invaded the tips of the corn roots under laboratory conditions as stated above.—Fernando Carvajal, 515 Nowlin Ave., Lawrenceburg, Ind.

4 Rapp, C. W. Aged bean seed, a control for bacterial blight of beans. Science 50: 568. 1919.

ERNEST ADAM DOPP 1896–1944

R. D. RANDS

The untimely death on September 24, 1944, of Mr. Ernest A. Dopp cut short a productive investigator in the prime of his career. Mr. Dopp was Assistant Pathologist in charge of the U. S. Sugar Plant Field Station at Canal Point, Florida. There he collaborated on sugar-cane breeding and the testing of varieties for disease resistance.

He was born September 12, 1896, at Superior, Wisconsin, the younger son of a family of horticulturists and gardeners. He graduated in 1917 from a teachers' training course at the Superior Normal School and was immediately drawn into the military service of World War I. Upon discharge, he taught for a year in high school and in 1920 entered the University of Wisconsin. There he obtained the B.A. degree in botany in 1922 and M.A. in botany and plant pathology in 1924. From 1924 to 1927 he was an instructor in botany at the University of Minnesota where he pursued some further graduate studies.

Dopp's service in the Bureau of Plant Industry, U. S. Department of Agriculture, extended from September, 1928, until his death; first, for two years in collaboration with the late W. W. Gilbert on joint investigations of cantaloupe and melon diseases, and from 1930 to 1940 with the present writer on diseases of sugar cane. He became an authority on identification of species of *Pythium* connected with root rots and made extensive studies of the mosaic virus and fungus leaf diseases, most of which remain unpublished. In 1940, he was transferred from Washington, D. C., to Canal Point, Florida, to take charge of the Department's sugar-cane breeding station where his cane disease studies along with varietal resistance tests were continued.

Dopp had a special gift in mathematics, which led him into extensive studies of statistical methods in agriculture and especially experimental designs for greenhouse and field tests. Because of his reluctance and even dislike of formal publication, this work might have been lost but for his unselfishness in communicating his ideas and results to associates.

Ernest Dopp exemplified to the highest degree those essential qualifications of modesty, generosity, an analytical mind, adept craftsmanship, and technical ability for effective teamwork and harmonious cooperative research. Thus, in the later years he became an indispensable member of the sizable group of federal and state workers devoted to the closely coordinated sugarcane improvement projects. Although results of work in which he was engaged are unsuited for frequent individual publication they have been none the less spectacular in the constantly increased yields of sugar cane throughout the Gulf States. His loss will be sorely felt.



ERNEST ADAM DOPP 1896-1944

He was author of the following publications:

An epidemic outbreak of red stripe disease of sugar cane and the reaction of some seed-ling progenies. 4th Cong. Internatl. Soc. Sugar Cane Technol. Proc. Bull. 46, 1932. (With R. D. RANDS.)

Variability in Pythium arrhenomanes in relation to root rot of sugar cane and corn. Jour. Agr. Res. [U.S.] 49: 189-221. 1934. (With R. D. RANDS.)

Influence of certain harmful soil constituents on severity of Pythium root rot of sugar cane. Jour. Agr. Res. [U.S.] 56: 53-67. 1938. (With R. D. RANDS.)

Pythium root rot of sugar cane. U. S. Dept. Agr. Tech. Bul. 666. 1938. (With R. D. RANDS.)

VIABILITY AND INFECTION OF LIGHT AND HEAVY COTTON SEEDS¹

C. H. ARNDT

(Accepted for publication April 20, 1945)

Chester² has published observations on the germination of acid-delinted cotton seed which had been separated into light and heavy seeds on the basis of their specific gravity relative to that of water. His generalized data indicate superior value for the heavy seeds. Similar methods have been used to separate sound from defective seeds in other plant species.³ This paper is a summary of studies made or supervised by the writer to ascertain the general applicability of Chester's observations to typical lots of cotton seed produced in the United States.

METHODS

The seeds were prepared by a method similar to that used by Chester. About 300 g. of seeds of each lot were acid-definted with concentrated sulphuric acid, after which the acid was removed by washing with water. During this process, but when not agitated, the seeds were separated into floating and submerged, or light and heavy, seeds. The two fractions were then air-dried and stored in the laboratory until it was convenient to test germination. The percentages of light and heavy seeds were ascertained from their air-dry weights.

Several methods were used to determine the percentage of germination. The 1934 lots of seed were germinated on paper toweling in covered trays at 30° C. In later studies, Ceresan-treated seeds were germinated in trays of steamed sand. For each test, 100 seeds regularly spaced and planted at a uniform depth were observed for 14 days or slightly longer.

When it was desired to ascertain the percentages of infected seeds, from 40 to 80 seeds of each sample of light and heavy seeds were germinated on sterile non-nutrient agar in test tubes, one seed to each tube. Immediately before germination, the seeds were surface-sterilized by immersion for 2 minutes in a solution of HgCl₂ in 50 per cent ethanol (2.5 g./1.), after which they were washed briefly with sterile water. Previous studies had indicated that Ceresan, which is commonly used to treat seed before planting in the field, could not be used, because of its adverse effect on germination when seeds are germinated on agar. The seeds were incubated for 14 days at 23° C. At the end of this period, notes were taken on germination; and the infecting fungi, when present, were identified. All data have been adjusted to a percentage basis for convenience in making comparisons.

² Chester, K. S. Gravity-grading, a method for reducing seed-borne disease in cotton. Phytopath. 28: 745-749. 1938.

³ Baldwin, H. I. Alcohol separation of empty seed, and its effect on the germination of red spruce. Amer. Jour. Bot. 19: 1-11. 1932.

¹ Technical contribution No. 123 of the South Carolina Agricultural Experiment Station. Investigation made in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

RESULTS

Relative Percentages and Weights of Light and Heavy Seeds. The results, as summarized in tables 1, 2, 3, and 4, show a range in the percentage of light seeds from 4 for the variety Half and Half to 94 for the variety Qualla (both in table 1).

The percentage of light seeds was relatively high in all lots of the variety Wilds, the lowest percentage being 47 (Table 1). The percentages for 9

TABLE 1.—Percentage germination, weight per 100 seeds, and percentage of light seeds for 18 lots of cotton seed of the crop of 1934, arranged in order of percentage of light seeds

Variety	State of origin	Weight per 100 seeds	Germination	Light seeds
The state of the s	and the control of th	Grams	Per cent	Per cent
Half & Half	Ga.	8.6	94	4
Cook	Ala.	9,0	86	5
Dixie Triumph	S. C.	9.8	61	13
Stoneville	Miss.	8.7	91	16
Mexican Big Boll	N. C.	12.5	93	16
DPL-11	Miss.	8.7	82	19
Dixie Triumph	S. C.	9.4	74	21
Cleveland	S. C.	9.3	58	23
Triumph	Okla.	10.5	65	24
Arkansas No. 17	Ark.	10.6	95	31
Super-7	S. C.	10.7	70	33
Missdel	Miss.	9.7	78	36
Farm Relief	S. C.	11.8	69	38
Wilds	S. C.	12.5	80	47
Startex	Texas	10.0	89	59
Rowden	Ark.	10.8	90	63
Acala	Okla.	11.0	89	69
Qualla	Texas	10.0	86	94

other lots of Wilds seed, representing the 1938 to 1941 crops, were 70, 80, 60, 94, 93, 84, 94, 80, and 63. The last 6 lots listed were grown in 1940 in different localities of northeastern South Carolina. The germination of the light seeds of this variety was generally high, or 75, 85, 90, 3, 88, 79, 69, 42, and 77 per cent, respectively, for the nine lots. The corresponding percentages for the heavy seeds were 90, 92, 92, 22, 80, 91, 84, 80, and 95. The low viability of the fourth lot was due to exposure to prolonged rainfall in the field before picking. The mean seed weight of the variety Wilds was generally high, as shown by the mean weights of 12.5 g. and 11.5 g. per 100 nongraded seeds (Tables 1 and 2).

That high seed weights tend to be associated with high percentages of light seeds in other varieties is indicated by the data of the several tables. Thus, if the lots listed in tables 1, 2, 3, and 4 are arranged into groups according to the mean weights of the seed, or weights of 8.0 to 8.9, 9.0 to 9.9, 10.0 to 10.9, and 11.0 to 12.5 g. per 100 seeds before flotation grading, the mean percentages of light seeds for each group will be 17, 27, 36, and 41, respectively. Similarly, although grown in the same county and presum-

TABLE 2.—Comparative data on sinkers and floaters in 12 lots of cotton seed produced in 1938

Wantaka and State	Light -	Weight of 1	100 seeds	Germ	ination	Light seeds	
Variety and State of origin	seeds	Heavy seeds	Light seeds	Heavy seeds	Light seeds	after vacuuma	
A STATE OF THE STA	Per cent	Grams	Grams	Per cent	Per cent	Per cent	
Mexican B.B., N. C.	. 6	11.6	12.4	91	82	58	
Farm Relief, S. C.		9.3	8.5	82	80		
Carolinadel, S. C.		9.8	8.2	90	65		
Acala, Calif.		12.3	11.7	89	82		
Dixie-Triumph, S. C.		10.1	9.6	93	68		
DPL, S. C.	. 27	9.7	8.4	96	77	Product.	
Miller, Miss.	27	10.6	9.5	91	69	******	
DPL, S. C	. 33	10.2	8.1	72	. 71	30	
Dixie-Triumph, S. C.		10.0	9.8	83	75	12	
Farm Relief, S. C.		10.0	9.6	96	84	12:	
Wilds, S. C.		12.6	11.0	90	68	28	
Acala, Okla.	81	7.9	7.0	34	14	11	

^a Percentage of light seeds of original separation which still floated after being subjected to a vacuum equivalent to 50 cm. of mercury while immersed in water.

ably from the same original seed stocks, the four lots of Coker 4-in-1 (Table 3) have mean weights per 100 nongraded seeds of 9.3, 9.9, 10.0, and 10.4 g., and the percentages of floaters are 10, 19, 23, and 50. Notable exceptions to the tendency of high seed weights to be associated with a high percentage of light seeds are shown by the Mexican Big Boll lots of high seed weight (Tables 1 and 2) and the Acala lot of low seed weight (Table 2).

TABLE 3.—Comparative data on heavy and light seeds in 20 lots of cotton seed produced in South Carolina in 1939

	T :1. 4	Weight of	100 seeds	Germi	nation	Weight of 100 embryo		
Variety	Light seeds	Heavy seeds	Light seeds	Heavy seeds	Light seeds	Heavy seeds	Light seeds	
See all of the second s	Per cent	Grams	Grams	Per cent	Per cent	d Grams	Grams	
Coker 4-in-1	10	9.5	7.3	88	73			
Marett-100	- 11	10.2	6.5	83	43	6.70	4.22	
Stoneville	11	10.7	7.6	64	41	and the second		
Stoneville	12	11.1	-8,6	67	79	and the second		
Coker-100	16	9.5	7.4	69	28			
Coker 4-in-1	19	10.3	8.1	80	57			
DPL	19	9.1	7.3	65	26		in the second	
White Gold		11.1	8.6	89	67	6.65	5.25	
Dixie-T	. 23	9.6	7.4	87	84	in in the second		
Coker 4-in-1	23	10.6	8.0	86	71			
Coker-100	28	9.9	7.4	87	44			
Dixie-T	31	9.9	8.3	60	24	6.61	5,68	
Stoneville	38	10.7	8.4	70	47			
Clevewilt	38	10.3	8,9	74	45	6.33	4.68	
Wonderwilt	39	11.1	8.8	82	46	Commence of		
Dixie-T	40	10.7	9.9	68	33	6,56	6,42	
Maretts-WR	4.4	10.6	10,4	80	38	7.12	6,26	
Dixie-T		9.4	9.1	52	23	6.18	5,73	
Clevewilt		10.4	9.3	51	7	and the second		
Coker 4-in-1		10.7	9,4	54	35	A month		

The weight of the heavy seeds was greater than that of the light seeds for all of the 37 lots listed in tables 2, 3, and 4, except for the first lot in table 2 and the Coker-100 lot of 1943 in table 4. The greatest difference between the weights of the heavy seeds and light seeds was that for the second lot in table 3, the weight of the latter seeds being only 63 per cent of that of the former. For 10 other lots (Table 3) the weight of the heavy seeds was at least 20 per cent greater than that of the light seeds.

In order to ascertain whether the low specific gravity of the light seeds might be partly due to occluded air, light seeds of six of the 1938 lots (Table

TABLE 4.—Comparative data on sinkers and floaters of acid-delinted seed used in field plantings in 1941 and 1943. Seed produced the preceding season

Year, variety,		337 • 3 .	Labora	tory tests
kind of seed, and State of origin	Light seed	Weight per 100 seed	Viable seed	No. seedlings diseased
And the state of t	Per cent	Grams	Per cent	1
1941 Plantingsa				
Coker-100, S. C.	21			
Heavy seed		9.2	91	0
Light seed		8.3	87	10
DPL-12a, Miss.	27			
Heavy seed		8.54	85	0
Light seed		7.11	81	1
Acala, Okla.	55			
Heavy seed			80	0
Light seed			80	Ö
1943 Plantings				
Coker-100, S. C.	66		0.0	
Heavy seed		11.74	99	18
Light seed		11.84	89	22
Delfos-651, Miss.	8			
Heavy seed		9.47	94	9
Light seed		8.61	66	9

^{*} These lots of seed were used in 19 plantings sponsored by the plant pathologists of the Southern States to evaluate the grading of acid-delinted seed. Detailed data will be published later by the supervising committee.

2) were subjected, while immersed in water, to a vacuum equivalent to approximately 50 cm. of mercury for three 2-minute periods, about 3 minutes elapsing between the successive vacuum treatments. After this treatment only 11 to 58 per cent of the original light seed still had a specific gravity less than that of water. This suggests that the difference between light and heavy seeds, in part, may be due to the greater amount of air occluded in the light seeds.

Since a small percentage of the light seeds did not contain embryos, it was thought likely that some of them might contain immature embryos and, consequently, that the proportion of the total seed weight represented by the weight of the embryos might be smaller in the light than in the heavy seeds. Relative weights of the embryos of heavy and light seeds were ascertained for 7 lots (Table 3). The embryos of these same lots of seed ranged from 60 to 67 per cent of the total weight of the seed for the heavy seeds and

from 52 to 68 per cent for the light seeds, with no clear tendency for the proportional weight to be greater for either class; but, for each lot the weight of 100 embryos of the light seeds was less than that for the embryos of the heavy seeds.

To ascertain the influence of the crop year on the relative proportions of light and heavy seeds, 14 lots of seed, representing 8 varieties, were collected in 1940 and 1941 from the same 4 counties of South Carolina—one county being selected as representative of each of the following sections of the State: northeastern, south-central, north-central, and northwestern. centage of light seeds for all lots of the same variety from the same locality was greater in 1941 than in 1940. A lot of the variety Wilds had the highest percentage of light seeds in both 1940 and 1941, or 64 and 85, respectively. For the other 13 lots, the respective mean percentages for the 1940 and 1941 lots were 9.7 and 14.3. For these same 13 lots in 1940, the lowest and highest percentages of light seeds were 5.3 and 15, respectively; and for 1941, 10.3 and 22, respectively; while the greatest difference between the percentage of light seeds in these two years for any one of these varieties from the same county was 8 per cent. Thus, although these data show that there may be variation in the precentage of light seeds from year to year for a given variety, the varieties tend to maintain the same relative order in a classification based on the percentage of light seeds.

One-half of each of the 14 lots of 1940 seed was delinted in September, 1940; and the remaining half was similarly delinted a year later, after storage in paper bags in the laboratory. The percentages of light seeds at both times were almost identical; thus, storage had no definite effect on the proportions of heavy and light seeds.

Germination of Heavy and Light Seeds. The germination of the heavy seeds for the 37 lots (Tables 2, 3, and 4) was equal to or greater than that of the light seeds, except for what might be a questionable instance for the fourth lot in table 3. Generally, the difference in germination between the two fractions was least when the germination of heavy seeds was relatively high. The lowest germination of light seeds relative to that of the heavy ones was 13 per cent (Lot 19, Table 3). For all 37 lots the mean germination of the heavy seeds was 62.5 per cent; that of the light seeds, 44 per cent.

Infection of Heavy and Light Seeds. Chester suggested that the removal of the light seeds might greatly reduce the incidence of disease in a cotton planting, since these seeds might be expected to be more highly infected by potential pathogens than the heavy seeds. For information on the relative infection, seeds of the two fractions of the 37 lots were germinated to ascertain the percentages of infected seeds and, when possible, the infecting fungi. As the differences in infection between the two fractions were about the same for all lots, the relevant data on infection have been summarized in table 5 without reference to the individual lots.

The heavy seeds were by no means necessarily free of pathogenic fungi, although the number infected was slightly less than in the case of the light seeds, except for the numbers infected by *Fusarium spp*.

The difference between them was small for Colletotrichum gossypii South, the most important of these infecting fungi. This fungus was found largely on seedlings and partially germinated seeds, as indicated in table 5, and was obtained in only a relatively few cases from the nongerminating seeds. In the latter, the presence of this fungus may have been obscured by other more rapidly growing fungi; for in the examination of nongerminating seeds, the first fungus to be identified was recorded as the fungus present, unless mycelial characteristics indicated the possible presence of other fungi. Thus, only one fungus was generally listed for a given seed, although the presence of other fungi cannot be excluded.

TABLE 5.—Relative infection of heavy and light seeds

	Num	ber of s			llings in bacteria		by vari	0118
Kind Total of No. seeds infected	Colletotrichum gossypii	Fusarium moniliforme	Fusarium spp.	Rhizopus sp.	Penicillium spp.	Diplodia theobromae	Miscellaneous fungi	Bacteria
Data for 1220 seeds of both fr	actions o	f the 37	lots in	tables	2, 3, a	nd 4		
Heavy 141 Light 264	53 69	17 32	30 29	0	1 8	$\frac{27}{70}$	10 33	8 12
Data for 500 seeds of both fra	ctions of	the 5 1	ots in ta	ıble 4				
Normal seedlings Heavy 20 Light 19	6 3	10 13	$\frac{1}{2}$	0	0.0	2 0	0 1	1 0
Partial germination (radica	ls formed	but no	cotyled	ons)				
Heavy 5 Light 25	3 9	$\frac{3}{7}$	0	0 3	0	$0 \\ 2$	0 3	0 = 0
Nongerminating seeds								
Heavy	$\frac{1}{2}$	0 5	$\frac{2}{7}$	3	0 8	0 8	0 5	4 5

Fusarium moniliforme Sheld. (Table 5) also occasionally caused lesions, ranging from small to extensively decayed areas on the cotyledons of seedlings. This fungus and Colletotrichum gossypii infected a number of the partially germinated seeds. The failure to form normal seedlings was due, at least in part, to infection by these two fungi. F. moniliforme appeared more frequently on nongerminating seeds than C. gossypii. Other Fusarium species, largely of the Gibbosum section, were occasionally found on seedlings, but more frequently on the nongerminating seeds, as were also such fungi as, Rhizopus spp., Penicillium spp., Diplodia theobromae (Pat.) Nowell (D. gossypina Cke.), miscellaneous fungi (in part Alternaria spp., Aspergillus spp., and fungi not forming spores while under observation), and bacteria. D. theobromae appeared more frequently on the nongerminating light seeds than on those of the heavy seeds.

Upon the suggestion of Dr. W. W. Ray that it might be possible to separate viable from non-viable seeds by appropriate water-ethyl alcohol mixtures, seeds of 5 lots of the variety Wilds (1940 crop) were flotation-graded in such alcohol solutions. There was a steady fall in the percentage of floating seeds for the five lots from 93, 94, 94, 80, and 73, respectively, in water; to 22, 43, 64, 43, 36, respectively, in 50 per cent alcohol; and to 8, 12, 25, 19, and 19, respectively, in 80 per cent alcohol. The percentage of alcohol necessary to obtain floating seeds of appreciably lower viability than those obtained in water ranged from 20 to 60. The mean germination of the seeds of these five lots which floated in water was 71 per cent; that of those which floated in 60 per cent alcohol, 54 per cent. Thus, although it may be possible to make a partial separation of viable and non-viable seeds by such solutions, this method appears to have no practical application.

SUMMARY

Seeds of lots of the upland varieties of cotton, Gossypium hirsutum L., were acid-delinted and then separated into two fractions, light and heavy seeds, on the basis of their specific gravity relative to that of water. After separation the fractions were dried, relative mean weights ascertained, and seeds of each germinated to ascertain their viability and infection by fungi and bacteria.

The light seeds in these lots ranged from 4 to 94 per cent, and the relative proportions of light and heavy seeds were determined more largely by varietal characteristics than by the viability of the seed, internal infection by fungi, or crop year. Varieties with high seed weights tended to have the highest precentages of light seeds. The weights of the embryos of the light seeds were less than those for the heavy seeds. The relative proportion of the total seed weight represented by the embryo was about the same for both. Removal of the air between the seed coat and embryo and the infiltration of this space with water changed most of the floating to submerged seeds.

The light seeds were somewhat more highly infected internally by fungi than the heavy seeds. The differences between the two in infection by the important seedling pathogens, Colletotrichum gossypii and Fusarium spp., were relatively small. The differences in infection were relatively large for such fungi as Penicillium spp., Diplodia theobromae, and Aspergillus spp.

The viability of the light seeds was generally less than that of the heavy seeds when the percentage of light seeds was small; but, when the percentage of the latter equalled or exceeded that of the heavy seeds, the viability of the light seeds approached that of the heavy seeds.

Improvement in seed quality which may be attained by water grading is determined by the characteristics of each lot of seed, and its general applicability for the improvement of seed quality is questionable.

DEPARTMENT OF BOTANY,

AGRICULTURAL EXPERIMENT STATION, CLEMSON, S. C.

VARIETAL VARIATION AND INHERITANCE STUDIES ON NATURAL WATER-SOAKING IN TOBACCO¹

HOWARD E. HEGGESTAD

(Accepted for publication April 28, 1945)

The predisposing effect of natural water-soaking² in leaf tissues to infection and development of tobacco wildfire [Phytomonas tabaci (Wolf and Foster) Bergev et al.] and blackfire [Phytomonas angulata (Fromme and Murray) Bergey et al. has been shown recently. Some variation in resistance had been reported previously in varieties of tobacco (Nicotiana tubacum L.) to these bacterial leaf-spot diseases, but varietal behavior under experimental trials had not been consistent, apparently because of wide differences in conditions provided for infection. Under field conditions, disease development may occur as a result of either storm water-soaking (7) or natural (internal or physiological) water-soaking (14). Varietal behavior toward disease may be expected to be different under these circumstances. One of the purposes of this study is to show that varietal variation in susceptibility to natural water-soaking occurs in tobacco, and that this is correlated with variation in susceptibility to the wildfire disease. Special efforts were made to study the inheritance of natural water-soaking after distinct varietal differences were found. The data presented are believed to be sufficient to show that the character of natural water-soaking is inherited in much the same way as many other quantitative plant characters, and that this character is closely associated with susceptibility to the wildfire disease of tobacco.

LITERATURE REVIEW

As far as is known, no previous work has been done on the varietal behavior and the inheritance of natural water-soaking in plants. The author has, however, recently presented (13) summarized results of some of the investigations reported in this paper.

Johnson (14) in 1936 first discussed natural water-soaking and its probable relation to infection and development of epidemics of blackfire and wildfire of tobacco. The following year, he (15) reported on further results with the artificial internal water-soaking method; that is, water-soaking by means of applying high water-pressure directly to cut stems or exposed roots. Many species water-soaked in this manner became infected easily when inoculated with weak pathogens such as the tobacco blackfire organism. Previous attempts to secure good infection with blackfire bacteria without water-soaking had failed even on tobacco. A close correlation was observed by Braun

congestion" suggested in a recent publication by Johnson (16).

¹ This paper was presented to the Graduate School of the University of Wisconsin in partial fulfillment of the requirement for the degree of Doctor of Philosophy. The writer is greatly indebted to Dr. James Johnson for advice and criticism during the work and the preparation of the manuscript.

2"Natural water-soaking" is synonymous with the more descriptive term "water-

and Johnson (5) between the amount of natural water-soaking found in farmers' plant beds and subsequent amounts of blackfire developing in the same plant beds. Johnson et al. (18) more recently reported, on the basis of observation, that natural water-soaking was apparently a genetically variable phenomenon and was therefore concerned with susceptibility to disease. It was on the basis of this observation that the present investigations were undertaken.

Proof of the bacterial origin of tobacco wildfire and tobacco blackfire was not established until 1918 and 1919. In the following decade, several investigators (3, 6, 8, 11) noted differences in varietal resistance to these leaf-spot diseases, but for the most part they were inconsistent or were regarded as of minor significance hardly worthy of consideration as a control measure in improving commercial varieties of tobacco. More recently, Garner et al. (12) reported that a selection of the Narrow-Leaf Orinoco variety grown in Virginia showed some resistance to blackfire. Johnson and Ogden (17) found the variety Havana 211 to be more susceptible to blackfire and wildfire than other local varieties following epidemics of the disease in the field; and Valleau et al. (19) reported Dark tobacco to be more resistant to angular leaf spot (blackfire) than Burley tobacco grown in the same plant bed. Hence, there is considerable observational evidence of varietal differences in resistance to bacterial diseases of tobacco. There is, however, a lack of consistent experimental evidence, and the reasons for this are more obvious now since there are two very distinct means of infection by the pathogens; namely, through "storm water-soaking" and "natural water-soaking."

METHODS AND MATERIALS

Methods. Tobacco seedlings that had been grown in steamed compost soil were transplanted, before the largest leaves were 20 mm. across, to 9 by 14-inch flats of steamed, low potash (2), sandy loam soil. About 2 dozen flats, each commonly planted with 10 plants of 3 different tobacco strains, were in each series. Two weeks after transplanting the tobacco had grown until the soil was almost covered; the seedlings were then ready for treatment. Extensive natural water-soaking in the most susceptible strains was induced by 24 to 48 hours in the moist chamber, from which the plants quickly recovered when returned to the greenhouse bench. One treatment each week for 3 consecutive weeks was usually made with the tobacco.

The moist chamber, located in a dark (1, 18) basement, had a fairly constant temperature that varied within only a few degrees of 65° F. during the entire greenhouse season. The relative humidity was maintained at 100 per cent by saturating the atmosphere with a fine mist from atomizers connected to an air line and kept in continuous operation. After sufficient exposure in the chamber, one flat of seedlings at a time was removed to a lighted room and the amount of water-soaking determined by examination of each plant.

A scoring system, comprised of 9 classes ranging from 0 to 4, was selected

as the most convenient method of recording the degree of water-soaking. The 4 major divisions or classes (scored 1, 2, 3, and 4) are illustrated in figure 1. Intermediate classes (scored 0.5, 1.5, 2.5, and 3.5) were also used, and plants with no macroscopically visible water-soaked areas on any of the leaves were scored 0. The 4 major classes may be described as follows:

Class 1. Water-soaking at the tip of one or more leaves, but largely confined to the leaf margin. The most heavily water-soaked leaf on the plant showing not more than an estimated 5 per cent of its leaf area affected.

Class 2. Water-soaking on one or more leaves, interspersed about most of the leaf margin and small amounts on other parts of the leaf blade. Approximately 5 to 20 per cent of the leaf area affected.

Class 3. Water-soaking as described for Class 2, except more of the leaf area (20 to 35 per cent) water-soaked.

Class 4. The most heavily water-soaked leaf, having numerous scattered water-soaked areas and an estimated 35 per cent or more of the leaf area water-soaked. Occasionally as much as 80 per cent of the leaf area was water-soaked under the conditions of the experiment. Leaves in classes 3 and 4 occurred infrequently under average experimental conditions.

TABLE 1.—Representative data showing arbitrary system of estimating natural water-soaking (score 0-4) on individual plants at three different times of exposure in moist chamber

Parent or	Consecutive exposures in		Plant and estimated water- soaking score						
F ₃ family	moist chamber		1	2	3	4	5	Ave.	
American (susceptible)	First One week later Two weeks later		2.5 2.5 1.5	$3.0 \\ 2.0 \\ 0.5$	$2.5 \\ 2.0 \\ 1.0$	$3.0 \\ 2.5 \\ 0.5$	$2.5 \\ 2.5 \\ 1.0$	2.7 2.3 0.9	
Daruma (resistant)	First One week later Two weeks later		$0.0 \\ 0.0 \\ 0.0$	$0.0 \\ 0.0 \\ 0.0$	$\begin{array}{c} 1.0 \\ 0.0 \\ 0.0 \end{array}$	$0.5 \\ 0.0 \\ 0.0$	$0.0 \\ 0.5 \\ 0.0$	$0.3 \\ 0.1 \\ 0.0$	
American × Daruma F ₃ 44	First One week later Two weeks later		1.5 1.5 1.0	$1.5 \\ 2.0 \\ 0.5$	$2.5 \\ 1.5 \\ 1.0$	$\frac{3.0}{1.5}$ $\frac{1.5}{1.0}$	$2.5 \\ 1.0 \\ 1.5$	$\frac{2.2}{1.5}$	
American × Daruma F ₃ 45	First One week later Two weeks later		0.0 0.0 0.0	$0.0 \\ 0.0 \\ 0.0$	$\frac{2.0}{1.5}$	$\begin{array}{c} 1.5 \\ 0.5 \\ 0.0 \end{array}$	$\begin{array}{c} 2.0 \\ 0.0 \\ 0.0 \end{array}$	$0.4 \\ 0.2$	

The detailed method of scoring is illustrated in table 1, showing representative data on 5 plants of each of 2 varieties and 2 F₃ families, together with the results of 3 consecutive exposures of the same series. The amount of water-soaking decreased with repeated exposures, but the same relative proportions were maintained between susceptible and resistant varieties or families. The figures presented in the other tables as water-soaking score or percentage of plants water-soaked represent the average behavior of the material in all exposures in the moist chamber.

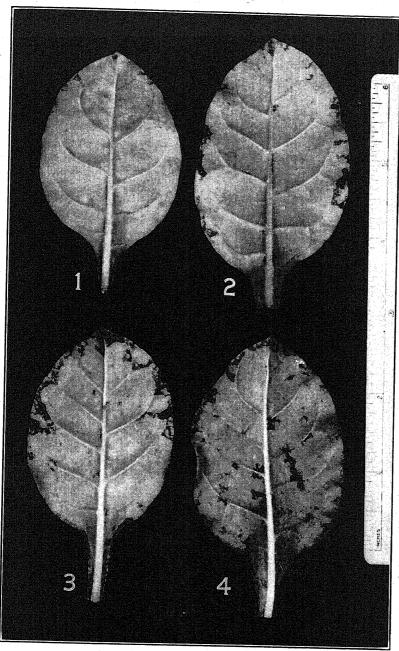


Fig. 1. Tobacco leaves illustrating arbitrary classes of water-soaking, as used in recording results. Numbers refer to classes of water-soaking.

Materials. All important local varieties and strains and representative strains of the more important domestic varieties of tobacco (Nicotiana tabacum L.) have been tested. In addition, 103 varieties and strains of tobacco, mostly South American, were secured through the cooperation of Dr. E. E. Clayton who had arranged for an extensive collection of tobacco seed by the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. Limited trials were made with 13 other species of Nicotiana. Several varieties of tomato (Lycopersicum esculentum Mill.), oats (Avena sativa L.), and corn (Zea mays L.) were kindly supplied by associates at the Wisconsin Experiment Station for other phases of the investigations.

EXPERIMENTAL RESULTS

Other workers (2, 18) have demonstrated that natural water-soaking in plants is influenced by the nature of the soil in which the plants are grown. Soils low in available potash favor natural water-soaking. In preliminary work on selection of a suitable soil, 50 small lots of soils from the sandy northwestern section of Dane County, Wisconsin, were compared as to their ability to influence water-soaking in tobacco plants. The maximum watersoaking (average score of 2.83) occurred with the sandy loam sample No. 23, and none appeared in plants grown in compost commonly used for other types of greenhouse experiments. In three soil samples, the sandy-loam K, silt-loam G, and the greenhouse compost, the available potash an acre amounted to 177, 715, and 3,030 pounds, respectively; and the average watersoaking scores for plants grown in these respective soils were 2.42, 1.92, and 0. Sandy-loam K was selected for most of the experimental work in the greenhouse. The soil was steamed in flats at 100° C, for 30 minutes to destroy damping-off organisms and weed seeds. The steaming also liberated some nitrogen and thus augmented the water-soaking in plants subsequently grown in the soil.

Older seedlings, in the greenhouse and in outdoor plant beds, are not water-soaked as easily as younger seedlings. Experimental plants were tested for water-soaking 3 times (Table 1) at weekly intervals beginning about two weeks after transplanting. In one comparison, involving 1080 plants, 91, 60, and 25 per cent of the plants water-soaked at 2, 3, and 4 weeks respectively after transplanting.

In outdoor tobacco plant beds under cloth covers, plants of all varieties may water-soak as soon as the first two leaves are unfolded and may continue to remain highly susceptible until the largest leaves are one inch or more in length. Some of the varieties classed as fairly resistant are rather susceptible as very young plants. However, as they become older, resistant varieties are rarely water-soaked under favorable weather conditions. Examination by Johnson and others (5, 18) have shown typical water-soaked areas on a variety of plants in various stages of maturity. For purposes of our investigation, it seemed desirable to limit the work and conclusions largely to young seedling plants.

Varietal variation in Nicotiana tabacum

The species *Nicotiana tabacum* contains hundreds of varieties or strains that are grown commercially in various parts of the world. Approximately 150 varieties and strains, including both domestic and foreign varieties, were grown in the seedling stage and compared for behavior in water-soaking. Out of these trials, 7 varieties were selected for further study as representing both extremes and intermediates in behavior. These were later compared in more critical tests as shown by summarized data from two experiments presented in table 2. The results of the two separate trials are in fairly close

TABLE 2.—The relative score and percentage of plants water-soaked in seven varieties and crosses between these varieties in the F_1 and F_2 generation

	Experi	ment 1a	Experi	ment 2b
Designation	Score	Plants water- soaked	Score	Plants water- soaked
	Average	Per cent	Average	Per cent
Parents				
American (susceptible)	2.36	100	1.69	88
Vastanhog	1.74	100	1.54	92
Wisconsin Seedleaf	1.40	80	0.64	45
Samsun	1.06	63	0.25	35
Havana 211	0.23	23	0.36	40
Havana 142	0.10	13	0.20	25
Daruma (resistant)	0.09	. 8	0.21	25
Crosses	F_1 gene	eration	\mathbf{F}_{2} gen	eration
American × Vastanhog	1.88	100	1.48	94
American × Wisconsin Seedleaf	1.53	100	1.58	91
American × Samsun	1.05	68	1.14	69
American × Havana 211	0.63	60	0.96	80
Vastanhog × Samsun	0.56	48	0.91	76
American × Daruma	0.41	58	0.92	73
Vastanhog × Daruma	0.79	68	0.72	70
Wisconsin Seedleaf × Daruma			0.72	66
Wisconsin Seedleaf × Havana 142	0.29	30	0.46	43
Samsun × Daruma	0.00	0	0.51	41
Havana 211 × Daruma	0.03	5	0.38	45
Havana 211 × Havana 142	0.00	0	0.18	26

a Two replications of ten plants each.

b Parents three replications, and F₂ progeny four replications of ten plants each.

agreement and seem to justify the belief that similar trials could be conducted throughout the greenhouse season with comparable results.

The two foreign varieties, American and Daruma (Fig. 2), represent extremes in susceptibility and resistance in greenhouse tests. In other experiments under conditions less favorable for water-soaking or when exposed for only a few hours to conditions favoring water-soaking, the variety American was not as extensively water-soaked as shown in figure 2, but the variety Daruma then failed to show any signs of water-soaking.

Similar differences in varietal susceptibility were also found in limited tests with half-grown plants of the varieties American, Wisconsin Seedleaf, and Daruma. Plants were grown in "lake sand" in 2-gallon glazed crocks

and supplied with modified (without potash) Hoagland's nutrient solution. Symptoms of potash deficiency were evident by the time the plants were transferred to the moist chamber. No marked differences were found in the degree of water-soaking between the two susceptible varieties American and Wisconsin Seedleaf which were both extensively water-soaked, but the resistant variety Daruma showed only slight water-soaking on 1 out of 12 plants tested. It is significant that the relatively large plants of these varieties behaved in the same manner as small seedlings in other tests.

In 1936 Dr. E. E. Clayton, of the Bureau of Plant Industry, found (correspondence) that the variety American was one of the most susceptible and

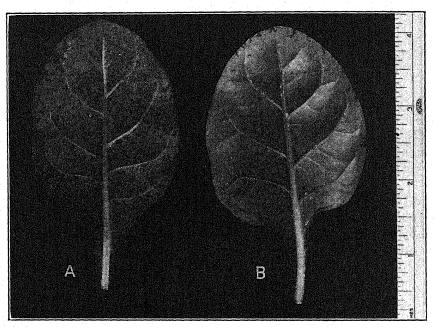


Fig. 2. Water-soaking of tobacco leaves showing extreme differences that may be secured under identical environmental conditions. A. American. B. Daruma.

the variety Daruma was the most resistant to storm water-soaking among approximately 100 varieties and strains of *Nicotiana tabacum* tested. The tests were made on nearly mature field-grown plants in connection with studies on resistance to disease (7, 12). The factors involved up to a certain stage may conceivably be similar to those concerned with natural water-soaking.

Species variation in Nicotiana

Fourteen species of *Nicotiana*, tested under moist-chamber conditions, varied in susceptibility to natural water-soaking from those very resistant but not immune to those which were nearly as susceptible as *N. tabacum*. The water-soaking pattern (that is, the size and distribution of water-soaked areas in the leaf) differed on some of these species. Consequently, it was

more difficult to make comparisons between these species than between the varieties of N. tabacum. For example, water-soaked plants of N. paniculata L. and N. glutinosa L. developed numerous small (many microscopic) water-soaked areas that were rather evenly distributed about the leaf. Water-soaked plants of N. tabacum L. and N. rustica L. developed, however, relatively large and conspicuous water-soaked areas that appeared first along the margin of the leaves. N. nudicaulis Wats., N. repanda Willd., and N. rustica L. were the most resistant of the species in our trials.

Inheritance of natural water-soaking

The F_1 and F_2 generation. The F_1 progeny from 11 crosses are compared in an experiment with an equal number of plants of the parents in table 2. The figures presented are the average calculations of two exposures in the moist chamber for 24 hours each, with a one-week interval between trials. For more ready comparison, the parents and progeny are arranged in the order of increasing resistance.

In the cross American (susceptible) and Daruma (very resistant), the F_1 generation was intermediate in susceptibility to water-soaking. The F_1 of the cross between the two most susceptible varieties, American and Vastanhog, was also susceptible. When the variety Samsun (intermediate) was crossed with the variety Daruma (very resistant), the F_1 progeny showed no water-soaking; whereas, some plants of the parental varieties water-soaked. Similarly, no water-soaking developed on the F_1 of the cross Havana 211 (intermediate) with Havana 142 (resistant). The failure to develop any water-soaking whatever in 2 trials on the F_1 of these two crosses is especially interesting in view of the fact that immunity is not involved in any of the parental varieties. This behavior suggests that the resistant character may be partially dominant. The results in the F_1 generation as a whole, however, indicate that the progeny are normally intermediate between the parents as far as such may be judged with a quantitative character.

Summarized results of an experiment with the F_2 progeny of the same crosses are also in table 2. The water-soaking scores for the different varieties do not cover as great a range as those with the F_1 progeny. This may be due in part to considerable variability between replications. The F_2 data are intended to show only that the amount of water-soaking in the F_2 generation of the several different crosses corresponds closely to that in the F_1 generation and to that which may be expected on the basis of parental behavior.

Although the results of the variety tests may be regarded as sufficient proof that the character of water-soaking is a heritable phenomenon, the limited F_1 and F_2 data in table 2 permit the conclusion that this character, although perhaps dependent upon many factors, is transmitted in crosses to succeeding generations according to expectations.

Good experimental data have not been secured for segregation or greater

variation in the F_2 than in the F_1 generation or in the parents. The number of plants that would be required would be impracticable for limited greenhouse space and moist-chamber facilities. It was believed that the time and space available might be more profitably applied to studies on the F_3 generation produced from selfed F_2 plants selected at random. Observational evidence on the individual F_2 progeny shown in table 2 was indicative of greater genetic variation in the F_2 than in the F_1 or in the parents. In the data this is only suggested by a relatively higher score and percentage of water-soaking in the F_2 than in the F_1 , or in the parental types, in crosses involving the more resistant parents.

The F_3 generation. Three of the most promising crosses were grown in the field in the F_2 generation to supply seed of selfed plants for testing in the seedling stage: American (susceptible) \times Daruma (resistant); American \times Wisconsin Seedleaf (intermediate); and Wisconsin Seedleaf \times Daruma.

TABLE 3.—The percentage distribution in 5 classes of water-soaking secured in tests on three parent varieties and 290 $F_{\rm S}$ families

Designation	Class (score) and percentage distribution ^a						
		0.25	0.75	1.25	1.75	2.25	
American P	100 plants	3	18	44	28	7	
Wis. Seedleaf Pa	100 do	20	36	41	3		
Daruma Pa	100 do	76	22	2			
\mathbf{F}_{a} progeny $(\mathbf{P}_{1} \times \mathbf{P}_{2})$	110 familiesb	0.9	21.8	47.3	29.1	0.9	
do $(P_1 \times P_3)$	95 do	13.7	40.0	33.7	11.6	1.0	
do $(\mathbf{P}_{a} \times \mathbf{P}_{a})$	85 do	31.8	43.5	23.5	1.2	*******	

a Based on the average of three scores from tests made at weekly intervals.

b Ten plants of each family tested.

Tests were made on approximately 100 F_3 families from each of the three crosses. Only 10 plants (selected at random) of each family could be tested, but this number was divided between two flats. It was necessary also to perform these tests in four separate series; consequently, one series was not strictly comparable with another. The three parental varieties were, however, used as controls in each series. The results of these tests are in a distribution table (Table 3) of average scores for the parents and the groups of families in each cross. In the F_3 generation, as in the F_1 and the F_2 , the parental influence is in the expected direction. Referring to the class-center score of 0.25 (high resistance), for example, only 3 per cent of the variety American and 20 per cent of Wisconsin Seedleaf, but 76 per cent of Daruma, fell into this class. Correspondingly, 0.9, 13.7, and 31.8 per cent of the three crosses fell into this class in the order of expectation.

On the basis of percentage distribution (Table 3), there is evidence of greater variability in the F₃ progeny of crosses between resistant and susceptible parents than in that of the respective parents. The data, however, are not strictly comparable for parents and progeny because the former are

based on individual determinations and the latter on the average of 10 plants. Nevertheless, there is a greater spread, with no peaks, in the ${\bf F}_3$ progeny of the two crosses involving the resistant parent Daruma than in the resistant parent itself where 76 per cent of the plants fell into the most resistant class (score 0–0.5).

These results are deemed sufficient to demonstrate that it should be possible to transfer the resistant characters to varieties of tobacco susceptible to natural water-soaking through breeding and selection.

Correlation between natural water-soaking and infection with tobacco wildfire

After it was evident that certain varieties of tobacco were more susceptible to natural water-soaking than others, it became important to determine whether these varieties were also more susceptible to wildfire under conditions favorable for the development of natural water-soaking. The results of two experiments with the three varieties, American (susceptible), Wisconsin Seedleaf (intermediate), and Daruma (resistant), are in table 4. Plants

TABLE 4.—Results of two experiments showing relative reaction of three varieties of tobacco to water-soaking, together with their relative susceptibility to wildfire infection following ineculation

Variety	Water-soaking scores			Number of wildfire lesions on a plant ^a		
	Exp. 1	Exp. 2	Ave.	 Exp. 1	Exp. 2	Ave.
American	2.35	2.60	2.47	6.9	30.2	18.5
Wisconsin Seedleaf	1.85	-1.85	1.85	4.5	20.9	12.7
Daruma	0.55	0.80	0.67	0.9	6.9	3.9

a Twenty plants of each variety in each experiment.

of these varieties were atomized from a distance of one foot or more with a water suspension of a pure culture of the bacteria, after which they were left in the moist chamber to permit water-soaking. The relative amounts of water-soaking developing after 48 hours' exposure in the moist chamber, and the relative amounts of wildfire (number of lesions) developing on the same plants 8 days from the time of inoculation, are shown in table 4. In these data, which include results of two separate trials, there is a direct correlation between the susceptibility of these varieties to water-soaking and their susceptibility to wildfire under the same conditions. In another similar trial, using plants two weeks older, most plants of the variety Daruma did not water-soak; whereas, all plants of both the variety American and the variety Wisconsin Seedleaf were rather heavily water-soaked. Most plants of Daruma likewise remained free of wildfire following inoculation; however, many lesions on a leaf developed on plants of American and Wisconsin Seedleaf. This difference in susceptibility is illustrated (Fig. 3) by representative leaves of the resistant variety Daruma and the susceptible variety American. The periphery of the leaf, which is usually the first to show evidence of water-soaking and often develops relatively large water-soaked areas, also develops the greatest number of and the largest wildfire lesions (Fig. 3, B). The correlation of water-soaking susceptibility and wildfire susceptibility was also evident from inoculation experiments with several species of tobacco. The results of these tests are not presented.

During the spring of 1943 and 1944, outdoor plant-bed experiments were conducted. Seed of several varieties were sown about May 1 in replicated 2 by 3-foot sections of the plant bed. The beds remained under muslin-cloth

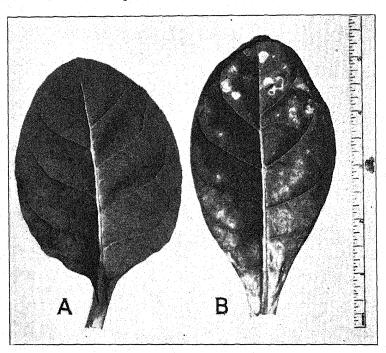


Fig. 3. Seedling tobacco leaves of two varieties, exposed to favorable conditions for water-soaking followed by artificial inoculation with the wildfire organism. A. Daruma, remained healthy. B. American, diseased.

cover most of the time, especially during periods of rain to reduce the possibility of storm water-soaking. The small tobacco seedlings were inoculated one month after sowing by watering with a suspension of powdered wildfire-infected leaf material.

The season in 1943, being unusually wet, proved to be very favorable for the trial. During the 3 weeks following inoculation on June 1 a total of 2.06 inches of rain fell, with at least a trace of precipitation during 14 days of this period. Records of relative humidity, both inside the covered plant bed and nearby outside, were taken from May 15 until June 16. A relative humidity between 95 and 100 per cent was maintained for at least 10 hours a day during 23 of the 32 days of the record as compared to only 2 days during the same period outside the plant bed. The longest period (51 hours)

of continuous, high relative humidity was recorded in the plant bed from 3:00 p.m. June 5 to 6:00 A.M. June 7, being continuously above 85 per cent and most of the time above 95 per cent in the absence of continued precipitation.

Extensive natural water-soaking occurred and numerous wildfire lesions began to appear on the more susceptible varieties about one week after inoculation. The severity of the disease gradually increased during the following two weeks. About 4 weeks after inoculation, when the notes (Table 5) were

TABLE 5.—Results in outdoor plant beds during 1943 and 1944, comparing 15 varieties of tobacco for relative susceptibility to water-soaking and wildfire following artificial inoculation. Average of 2 replications

		1943	. •		1944	
	-	Wi	ldfire		Wi	ldfire
Variety	Water- soaking score (40 plants)	No. lesions on a leafa (20 plants)	Diseaseb score (20 plants)	Water- soaking score (40 plants)	No. lesions on a leaf ² (100 plants)	Percentage diseased plants (200 plants)
Wisconsin Seedleaf (Strain T) American Vastanhog White Burley (Judy's Pride) Pennsylvania Broadleaf	1.1	24.0 34.1 28.2 14.1	100.0 46.4 54.4 32.1	1.9 1.4 1.4 1.4	14.4 6.9 8.5 4.3	100.0 99.5 97.5 90.0
(Slaughter) Maryland Broadleaf Turkish (Samsun) Maryland Mammoth	$\begin{array}{c} 0.8 \\ 0.7 \end{array}$	$10.9 \\ 28.9 \\ 1.2 \\ 13.9$	18.2 36.6 1.2 79.9	0.3 0.3 0.5	$1.6 \\ 4.6 \\ 7.5$	71.0 93.0 98.5
Connecticut Broadleaf Var. atropurpurea Havana 211	$0.6 \\ 0.5 \\ 0.2$	$19.0 \\ 10.4 \\ 26.2$	39.7 16.0 44.6	0.9 0.9 0.6	6.9 7.3 15.8	97.0 98.5 100.0
Havana 142 Yellow Pryor Orinoco (White Stem) Daruma	0.1	14.9 1.5 2.0 1.8	$20.9 \\ 1.6 \\ 2.3 \\ 2.4$	0.0 0.0 0.0 0.0	7.1 1.4 1.6 1.3	97.5 53.0 64.0 52.0

a Counts made only on the leaf with greatest number of distinct lesions (halos).

taken, plants of certain varieties were either killed or severely stunted and other varieties had just a few isolated lesions. The lesions were counted on single leaves from 20 plants of each variety and a disease "score" computed which also included an estimate of the amount of necrosis on these plants. The score for the most susceptible variety was then placed at 100 and the other scores were adjusted accordingly. The notes on water-soaking (Table 5) were taken 11 and 14 days after inoculation. Water-soaking occurred on other days, but detailed varietal comparisons were not made.

The 1944 plant-bed experiment was conducted in the same way as in 1943 except that the bed was located on muck soil rather than on silt loam soil and the young seedlings were inoculated 3 times at 4-day intervals rather than just once. The relative susceptibility of the varieties tested, both to water-

^b A score based on number of leaves on a plant completely destroyed by the disease and on percentage of leaf area necrotic as well as on number of lesions.

soaking and to wildfire, was approximately the same in the two years. The varieties Daruma, Orinoco, and Yellow Pryor proved to be very resistant to both water-soaking and disease. These varieties in 1943 and in 1944 developed only about 1/20 and 1/10, respectively, the number of lesions found on the more susceptible varieties (Table 5). About 44 per cent of the plants of these resistant varieties were free of disease in 1944; whereas, 100 per cent of the plants of Wisconsin Seedleaf and Havana 211, another Wisconsin variety, were diseased.

The results secured on tobacco varieties with wildfire are believed to be equally significant in relation to varietal differences in resistance and susceptibility to the closely related (4) blackfire disease under similar environmental conditions.

Variation in varieties of other plant genera

Tomatoes. A comparison was made on the water-soaking behavior of 17 commercial varieties or strains of tomato under moist-chamber conditions.

TABLE 6.—Results showing the relative susceptibility of seven varieties of tomatoes (Lycopersicum esculentum) to water-soaking when placed under favorable conditions for its expression

Variety	Water-soaking scorea			Percentage of plants water-soakeda		
	Exp. 1	Exp. 2	Ave.	Exp. 1	Exp. 2	Ave.
Landreth's Bloomsdale	2.6	2.2	2.40	100	100	100
Golden Globe	2.4	2.1	2.25	100	100	100
Clark's Early	2.4	1.8	2.10	100	94	97
Early Baltimore	1.9	1.8	1.85	100	100	100
Harris' Extra Early	0.8	1.5	1.15	83	94	89
'Pearson'	0.9	1.1	1.00	92	88	90
Snow Ball or Albino	0.8	0.9	0.85	64	81	73

^{*} Data based on two exposures in chamber, with six to eight plants of each variety.

In a preliminary experiment, 6 potted plants, 8 to 12 inches in height, of each variety were compared, using a sandy loam soil low in available potash. Later, 8 potted plants of each of 7 varieties—those showing the greatest range of variation in the first experiment—were further tested using compost soil, which is not as favorable to natural water-soaking as soil of lower fertility. Two tests were made with each group of plants at 10-day intervals and the results averaged.

Summarized data are in table 6 for the 7 varieties included in both experiments. The variety Bloomsdale was the most susceptible, with an average score of 2.40; and the variety Snow Ball was the most resistant, with an average score of 0.85. The percentage of plants water-soaked varied only from 100 to 73 per cent for the two varieties respectively. Varieties of tomato are, in general, more susceptible to natural water-soaking than are varieties of tobacco.

Oats. Preliminary trials were made on the relation of natural water-

soaking to infection with halo-blight [Phytomonas coronafaciens (Elliott) Bergey et al.] of oats in the seedling stage. Six varieties or selections were used, representing a range of reaction to the disease on the basis of field observations by Dr. H. L. Shands of the Dept. of Agronomy at the Wisconsin Station.

Twenty-five seeds of each variety were planted in rows across each of 6 flats (14 by 20 by 5 inches) containing a 3:1 mixture of greenhouse compost and sand. Oat seedlings were easily water-soaked, sometimes within an hour after placing in the moist chamber. Water-soaking was also observed, when the air and soil moisture were sufficiently high, on plants placed outside. Four of the replicated flats were inoculated by atomizing with a water suspension of bacteria at a distance of one foot or more from the seedlings so that the organisms should not be forced into open stomata.

Distinct varietal differences were found both in susceptibility to watersoaking and to disease. Summarized results are in table 7. The average

TABLE 7.—Results showing the relation of water-soaking to susceptibility to haloblight (Phytomonas coronafaciens) of varieties and selections of oat seedlings

	Water-	Halo-bligh	t reaction
Designation	soaking score (average)	In greenhouse following artificial inoculation	In field following natural infection ^a
X217	3.8	Very susceptible	Susceptible
Marion	3.5	Susceptible	Susceptible
X216	3.0	Susceptible	Moderately resistant
Vicland	2.5	Moderately susceptible	Moderately susceptible
C.I. 4007	1.4	Resistant	Resistant
C.I. 4006	0.9	Resistant	Resistant

a Rating by Dr. H. Shands of Department of Agronomy.

water-soaking score, using the range of 0 to 4 as for tobacco, varied from 0.9 for the variety C.I. 4006 to 3.8 for the variety X216, and resistance to disease was directly correlated with resistance to water-soaking. The lesions on the resistant varieties were smaller and fewer as compared to lesions on the susceptible varieties, some of which were very elongated, corresponding somewhat to previous water-soaked areas on the same leaves. The varietal reactions following artificial inoculation were approximately the same as those observed by Dr. Shands following natural infection (Table 7). The results suggest a reliable and rapid means of selecting and developing varieties resistant to the halo-blight disease.

Corn. Preliminary trials on water-soaking were also conducted with 17 dent-corn inbreds, 8 of which were selected to represent the range of susceptibility to Stewart's wilt [Phytomonas stewartii (Smith) Bergey et al.] as reported by Elliott (10). Twenty plants of each inbred were grown in flats for these trials.

Corn seedlings are very susceptible to natural water-soaking. Frequently, seedlings water-soaked in the greenhouse without being in the moist

chamber; whereas, this rarely occurred with tobacco, tomato, or oats. Differences in susceptibility between inbreds were not easily determined. Numerous droplets of moisture adhered to the leaves, making observations more difficult and uncertain, and water-soaking was somewhat obscured by the dark green color of certain inbreds. Consequently, the data on varietal variation in corn are not presented in detail. More data would be required to determine which inbred is most resistant to water-soaking.

Two of the inbreds, KYS and 32B, were consistently more susceptible to water-soaking than were the others. KYS is the most susceptible to wilt of 50 selections that were tested by Elliott (10). Leaves of apparently healthy plants of these two varieties water-soaked so frequently, just standing on the greenhouse bench, that they became necrotic. This necrosis may have been associated with the entry of weak parasites into water-soaked tissue as suggested by Johnson (15) for other plants, or it may be due to an accumulation of toxic concentrations of salts following frequent guttation and drying as suggested by Curtis (9).

Differences in susceptibility to water-soaking of inbreds, with both seedling and nearly mature plants, have been observed on plantings made outdoors. For example, in one trial with 40 seedlings of each of two inbreds, no plants were water-soaked of the inbred Ohio 67, which is moderately resistant to wilt; whereas, all plants were water-soaked of KYS, which is very susceptible to wilt.

The results, although limited in extent, suggest that water-soaked tissues may influence infection with and development of the Stewart's wilt disease.

DISCUSSION

The results have demonstrated that varieties of Nicotiana tabacum and other plants possess varying degrees of resistance to natural water-soaking. The variation secured was of such magnitude that it was believed to offer opportunity for the study of the inheritance of this character in plants. It seemed especially desirable to determine whether resistance to watersoaking might be introduced into susceptible commercial varieties with the purpose of improving their resistance to specific diseases. A part of the present investigations was to determine the correlation between natural water-soaking and resistance to disease, especially as applied to tobacco wildfire. Fairly good agreement was found, which not only supports the earlier conclusions from this laboratory that water-soaking predisposes plants to disease, but suggests a shorter and perhaps improved method for selecting plants for disease resistance. It may be easier, for example, to eliminate varieties or plants susceptible to certain diseases by first determining their relative susceptibility to water-soaking. The technique may be particularly useful in breeding for resistance to various bacterial leaf-spot diseases that occur infrequently in natural epidemics or that are difficult to reproduce experimentally in the greenhouse. The major contribution of this investigation is, therefore, the suggestion it offers for continued efforts with numerous other hosts and diseases.

Considering the entire data on the F_1 and F_2 generations of twelve crosses between tobacco varieties varying in response to natural water-soaking, as well as the results on F_3 progeny of three of these crosses, the genetic behavior is best explained on the basis of multiple factors segregating in a normal manner. There is some evidence, however, of the existence of dominant factors for resistance. The most significant fact from the genetic studies is that crosses may be made with commercially important susceptible varieties with some assurance that lines resistant to water-soaking may be secured.

Some difficulties and problems arise in an investigation of this sort involving a special technique to secure the expression of a genetically new character that is easily influenced by non-genetic factors. There is no certainty, for example, that the varieties used were homozygous for the character studied, or that a very considerable part of the variation secured within a variety is a normal variation due to uncontrollable variation in the environment. It is certain that the expression of the natural water-soaking character is very delicately balanced with the environment. Consequently, minor modifications may prevent its appearance and greatly alter the severity of disease.

Questions of proper or satisfactory terminology are also justifiable in connection with the genetic behavior of natural water-soaking. The terms "resistance" and "susceptibility" to natural water-soaking have been used in this paper. This terminology is in a measure confusing when it is used in close association with their long-established use as applied to genetic variations in host behavior toward pathogens. Proper terminology for future use is therefore sought.

SUMMARY

The natural water-soaking reactions of several foreign, domestic, and local varieties of tobacco have been compared under moist-chamber and outdoor plant-bed conditions. The amount of water-soaking is expressed as (1) a "score" based on the amount of leaf area water-soaked, and (2) the percentage of plants water-soaked. One moist-chamber test yielded, for example, differences in score ranging from 2.36 to 0.09 and in plants water-soaked from 100 to 8 per cent respectively. The susceptibility of varieties was greatly influenced by environmental factors; however, the relative differences between the varieties remained approximately the same.

The inheritance of the natural water-soaking character in crosses involving seven varieties ranging in reaction from the highly resistant to highly susceptible has been studied. The F_1 progeny of most crosses were apparently intermediate between the parents; however, when some varieties were crossed, there was evidence of partial dominance of resistance. The reactions of the F_2 and F_3 families clearly indicate segregation of genetic factors controlling the inheritance of this character. Many genetic factors are apparently involved. F_3 families approaching the resistance of the most resistant parent were obtained.

Varieties of tobacco likewise showed varying degrees of resistance to wildfire (Phytomonas tabaci) when inoculated under conditions favorable for the occurrence of natural water-soaking. Results in greenhouse and moistchamber experiments were essentially the same as in experiments in outdoor plant beds. Resistance to this disease was closely correlated with resistance to water-soaking.

Variation in reaction to natural water-soaking was also found between different species of Nicotiana and between varieties of tomato, of oats, and of corn. Varieties of oats inoculated with the halo-blight organism also showed correlation of water-soaking and disease.

WISCONSIN AGRICULTURAL EXPERIMENT STATION, MADISON, WISCONSIN.

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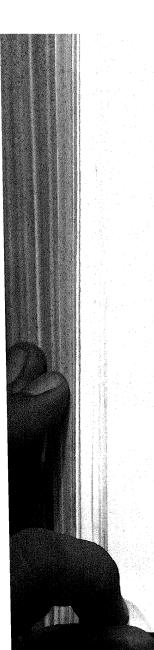
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THE NEMATOCIDAL AND FUNGICIDAL VALUE OF D-D MIXTURE AND OTHER SOIL FUMIGANTS

G. K. PARRIS

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Until recently, the most effective soil fumigant, other than steam, for the control of the root-knot nematode, Heterodera marioni (Cornu) Goodey, and of most soil fungi, was chloropicrin. Taylor (6) has reviewed the literature pertaining to control of nematodes by this material and Leukel (2) lists a number of investigators who have found chloropicrin to be an effective fungicide. In April, 1943, Carter (1) reported on a new material, D-D mixture for short, which had shown great promise as a "soil amendment and disinfestant" in the Hawaiian Islands. Three years' testing under tropical conditions showed that D-D mixture injected in soil at intervals one foot apart, at 150 to 200 pounds per acre, without benefit of subsequent cover, gave good control of a soil complex including Anomala beetle larvae (Anomala orientalis), the root-knot nematode (H. marioni), and Pythiaceous fungi (presumably Phytophthora cinnamomi, P. parasitica, and other members of the Pythiaceae that attack pineapple in Hawaii). Carter considered that D-D mixture was at least the equal of chloropicrin in its benefits and, furthermore, it was lower priced and simpler to handle. Pinckard (4) subsequently reported the value of D-D mixture as a nematocide.

EXPERIMENTS

The experiments reported here were begun in July, 1943, to test the nematocidal and fungicidal properties of D–D mixture and were continued in their various phases until September, 1944. Both forms of D–D mixture were studied, the redistilled mixture of approximately two parts of 1,3-dichloropropylene and one part of 1,2-dichloropropane, and the crude mixture of approximately two parts of 1,3-dichloropropylene, one part of 1,2-dichloropropane, and one part of 3-carbon-atom compounds of trichlorides and tetrachlorides. In addition two other materials similar in chemical nature, monochlorobutenes and trichlorobutanes were studied. These materials were supplied through the courtesy of their manufacturer, the Shell Development Company, Emeryville, California. In preliminary tests, chloropicrin¹ was compared with the other products, but in later trials it was not included.

The root-knot nematode (Heterodera marioni) was the only nematode studied; fungi studied were Rhizoctonia sp. (probably R. solani), Fusarium oxysporum f. lycopersici (Sacc.) Snyder and Hansen, Fusarium sp. (probably F. martii) and Pythium aphanidermatum (Edson) Fitzpatrick.

The soils used varied from Norfolk loamy sand, well drained, with or ¹ The chloropierin was supplied by Innis, Speiden and Co., 117 Liberty Street, New York, as "Larvaeide."

without a fairly high organic matter content, to Woodstown silt loam. In a single test, a silt loam, which had been used in greenhouse culture for 8 months and was well mixed with rotted leaves, was studied. Experiments were performed outdoors during the fall of 1943, the winter of 1943-44, and the spring and summer of 1944, as well as in a greenhouse during the fall of 1943 and winter of 1943-44.

In early outdoor tests and in all greenhouse trials the D-D mixture, or other materials used, was injected in holes 3-4 inches deep (made by hand with a sharp stick), one hole in the center of each square foot of surface. The points of application were not staggered. This method is slower than the standard procedure described by Taylor (5), because the application points are more numerous and the rate of chemical per injection hole is less for an equal poundage of chemical per acre. In later tests, Taylor's spacing and rate of chemical per injection hole were adopted; the injections were 12 inches apart in staggered rows, the rows 12 inches apart, the holes 6 inches deep. In still other tests, the rows were 18 or 36 inches apart with injection points in the row 12 or 18 inches apart.2 The chemicals were measured and injected by hand, using a burette and a flask with a side tube. With one exception, that of a trial in which a chloropicrin-treated plot was compared with a D-D-treated plot (Experiment No. 1), the treated soil was not covered; sometimes the soil was well wetted down after treatment, sometimes it was left undisturbed.

Crops, either seeded directly or as transplants, were started in out-door treated soil 2-3 weeks after chemicals were applied, or as soon as convenient for cooperating farmers on whose lands the tests were installed. In the greenhouse, pea and spinach seed, the two test plants grown, were sown at intervals ranging from 1 to 21 days after the soil was treated. All greenhouse tests were in raised benches, one foot deep, 12 feet long, and either 3 or 6 feet wide.

The control of root-knot achieved by the D-D mixture or other soil amendments was judged either visually, by comparing the roots in gross characters with plants grown alongside in untreated soil, or by actual count of the number of galls on representative plants grown in treated and untreated soils respectively. Soil temperatures at 6-inch depths were taken when feasible.

Studies on Root-Knot

Experiment No. 1 consisted of 18 plots, each 3 by 5 feet, arranged linearly in a concrete container 6 feet wide, 2 feet deep, and 75 feet long, filled with a silt loam mixed with rotted leaves as used in a greenhouse in which cucumbers and tomatoes had been grown during the preceding winter and spring. This soil was heavily infested with Heterodera marioni. The chemicals, D-D mixture (both crude and purified), chloropicrin, monochlorobutenes, and trichlorobutanes, were injected into 3 replicated areas of 15 square feet each, and the treated areas were separated by an untreated space one foot wide

2 Suggestion from G. H. Godfrey; personal correspondence.

to hinder, if not prevent, inter-plot diffusion of the fumigants. Injections were at the rate of 150 pounds per acre in holes 3 to 4 inches deep, one injection point for every square foot. Appropriate areas left untreated served as checks. After treatment the surface soil was wetted down, and, in addition, plots with chloropicrin injections were covered with a double layer of wet gunny sacking. Plots were treated on July 1-3 (1943), and two weeks later tomato seeds were sown. The odor of the D-D mixture was still noticeable then, that of the other 3 chemicals could not be detected. Germination was good and there was no evidence of any toxic action by the D-D mixture. Seven weeks later (Sept. 3-4) the tomato plants were dug, the roots were examined for nematode galls, and the tops were weighed. Galls were not counted, but good control of the nematode by crude and by purified D-D mixture and by chloropicrin was obtained. No control was obtained with monochlorobutenes and trichlorobutanes. Check plants were severely galled. Mean weights of tomato tops (in grams) were 58 each for the check and monochlorobutenes, 78 for trichlorobutanes, 85 for purified D-D mixture, 99 for crude D-D mixture, and 114 for chloropicrin. (The difference necessary for statistical significance is 25 grams.)

Experiment No. 2 is reported here despite the fact that the soil used in Experiment No. 1 was used over again, a procedure not beyond criticism. The soil was removed from the container, well mixed, and replaced. On September 18 it was treated with D-D mixture, crude and purified, and with chloropicrin, 180 lb. per acre. Each plot had 25 square feet of soil and one injection point per square foot, and each treatment was in 3 replicates. Again, appropriate areas served as checks, and the treated areas were separated from each other by an untreated space one foot wide. As in Experiment No. 1, the soil was wetted down, but in Experiment No. 2 no plots were covered. Bush beans were selected as test plants, because of the advanced season, and sown in each replicate at 3 different dates, 11, 16, and 18 days after soil treatment. Sash were placed over the plots 6 days after the last planting and maintained in situ with appropriate regulation to suit outside temperatures. One month after the first planting the plants were dug and counted, and the roots were examined for root-knot. The data obtained are in table 1.

D-D mixture again gave good control of the root-knot nematode, but it caused some injury (expressed as reduced emergence³) to beans planted as long as 18 days after the soil was treated. All chloropicrin fumes apparently had been released at 16 days, but not at 11 days, after treatment.

Experiments No. 3 and No. 4 were in the same soil, in the same container, as Experiment No. 2. The 12 plots of Experiment No. 2 were left untouched,

³ In another outdoor test, D-D mixture and trichlorobutanes, injected 6 inches deep into soil at 190 and 380 pounds per acre respectively, have injured tomatoes when transplanted 14 days after treatment. The soil was a poorly drained Woodstown silt loam of relatively low organic matter content and pH 5.5; the mean minima and mean maxima soil temperatures, 6 inches deep, were 71° and 80° F. respectively. No injury was noted in this soil when monochlorobutenes were simultaneously injected at rates up to and including 1,000 pounds per acre.

TABLE 1.—Relative degree of control of Heterodera marioni in infested soil treated with D-D mixture (crude and purified), with chloropicrin, or left untreated. Rate of application, 180 pounds per acre. Indicator crop was bush bean

	Treatment of soil	Nematode attack	Germination of beans sown at different times after treatment of soil		
			11 days	16 days	18 days
			Pct.	Pct.	Pct.
	Untreated	Severe	82.5 ± 6.1	84.3 ± 4.3	82.6 ± 4.3
	D-D (crude)	Slight to moderate	68.0	77.7	74.5
	D-D (purified)	Slight to moderate	64.0	79.5	76.0
	Chloropicrin	Moderate	65.0	89.2	83.6

with weed growth unhindered and abundant, until February 10, 1944, when weeds were pulled, and the soil chopped with a hoe, care being taken to keep the soil in each plot in place. On February 25, Irish potatoes were planted, 20 per replicate, and grown without benefit of fertilization until harvested on June 13. On May 9 potatoes growing in soil into which D-D mixture had been injected were darker green and of more uniform size than plants growing in control soil. The heights of all plants were taken; plants growing in soil treated with D-D mixture averaged 11.5 inches, while those in untreated soil averaged 8.5 inches, a difference that is statistically significant. Plants growing in chloropicrin-treated soil averaged 10.8 inches tall. There was little difference in yields of tubers from different plots; nematode infection was light in check plots and relatively sparse on tubers from treated plots. The roots themselves were not examined.

In Experiment No. 4, four rows of Lima beans, 25 seeds per row, were planted on June 18 in each plot used in Experiment No. 3. The soil had been kept in place except for the small amount of hoeing necessary to prepare a seed bed. On August 8, eight representative plants were removed, with the roots intact, from each plot, the soil was washed away, and the nematode galls were counted. The counts (Table 2) show that for 9 months,

TABLE 2.—Effect of treating root-knot menatode infested soil with D-D mixture and with chloropicrin; mean number of galls on roots of Lima bean plants grown in treated and untreated soil, nine months after soil was disinfested. Eight plants from each plot were examined

Plot No.s	Treatment of soil	Mean number of nematode galls	Plot No.	Treatment of soil	Mean number of nematode galls
1	D-Db	0.3	7	Chloropicrin	238.3
2	None	524.3	8	None	325.5
3	D-D	0.0	9	D-D	30.7
4	Chloropierin	44.1	10	Chloropicrin	149.0
5	D-D	0.3	11	D-D	3.3
6	D-D	0.0	12c	None	

* Plots 1 and 2 were at one end of the concrete container.

b Both crude and purified D-D mixtures were used; since results were the same with both, no distinction is indicated.

c Plot 12, an untreated check, was abandoned because of its proximity to a drainage pipe that caused flooding of the plot.

with no precautions taken to prevent interplot movement of soil by rain or irrigation water, and with weed growth unhindered for at least 4 months, a root-knot nematode population, reduced by the use of D-D mixture, had maintained its low level. Where chloropicrin was used, the nematode population, once markedly reduced, had increased, but had not reached its former level. It should be noted here that the manufacturers of chloropicrin generally recommend that their fumigant, when used against nematodes in the soil, be applied at a much higher rate per acre than was used in these tests.

Experiment No. 5 consisted of long, paired beds of well-drained Norfolk loamy sand, with moisture-holding capacity approximately 11 per cent, pH approximately 6.5, and organic matter content high and so maintained for the past 20 years by the owner of the land. Root-knot had caused some losses in this general area in 1943 (3). The beds were made up for celery, to be grown under sash. Each bed was 4 feet wide and over 200 feet long; in these tests 90 linear feet per bed were used. The 3-foot space between each pair of beds was occupied by the irrigation pipe of a Skinner irrigation system, and two beds not included in the experiment separated each pair of One bed of each pair was treated with D-D mixture, there beds studied. being 3 rates of application per bed, 120 square feet of soil for each application rate. The untreated bed of each pair served as check. On January 26 or on February 16, 1944, the D-D mixture was injected at depths of 4.5, 7, and 8 inches, at points 12 inches apart in staggered rows, and injections were at rates of 125, 190, 200, 250, 325, and 400 pounds per acre. was left uncovered until March 13-15, then celery transplants, free from Heterodera marioni, were set out, and sash were placed in position. sash were regulated to suit outside temperatures until permanent removal on April 20, 1944. The actual temperatures at 6-inch soil depth in these months are not known, but we do have access to soil temperatures at this depth, taken in the same area, for a 5-year period, 1932 to 1936. Mean soil temperatures for January to May inclusive may have been approximately as follows, except that the soil temperatures would have been higher as soon as sash were placed over the celery plants: January, maximum 38° F., minimum 34° F.; February, 40° F. and 36° F.; March, 47° F. and 41° F.; April, 64° F. and 57° F.; and May, 81° F. and 72° F.

No injury that could be ascribed to the presence of the D-D mixture was noted. On June 6, when the grower on whose land the test was installed was harvesting his crop, 60 plants were pulled from the center of each treated area and a similar number from the adjacent check plot, the soil was shaken from the roots, and the celery was weighed without trimming. Five other plants, chosen at random, were removed at the same time from each treated and untreated area, with care to preserve the roots as intact as possible. The soil was washed from the roots, and the nematode galls of each root system were counted. Results (Table 3) show that D-D mixture reduced the nematode population, but the yields from treated and untreated areas of soil are little different.

Experiment No. 6 was in the same type of soil and on the same farm as Experiment No. 5, the tests being 200 yards apart. The area was one in which celery was diseased severely with root-knot in 1943, yields being reduced 22–36 per cent in untrimmed plants and 24–48 per cent in trimmed celery (3). Two rectangular and parallel areas of infested soil, each 4 feet wide and 150 feet long, on either side of an irrigation pipe of a Skinner irrigation system and separated by a 4-foot width of soil, were selected, and into one strip D–D mixture was injected on February 26, 1944, while none was injected into the companion strip. The method of application was as described for Experiment No. 5, with rates of application 210, 290, and 375

TABLE 3.—Average numbers of nematode galls on, and yields of, celery grown in soil infested with Heterodera marioni and treated with D-D mixture or left untreated

Treatment (pounds D-D mixture per acre)	Depth of application (inches)	Date of application (1944)	Mean number galls per plant	Mean yield per plant (pounds)
125	7	Jan. 26	36.1	1.16
0			114.1	1.17
190	7	do	8.0	1.04
0			490.1	1.13
250	7	do	0.9	0.77
0			365.2	1.07
200	8	Feb. 16	4.2	0.78
0			> 1000.0a	0.77
325	8	do	0.0	0.81
0			273.2	0.92
400	8	do	0.0	1.15
0			820.4	0.92
200	4.5	do	0.2	0.86
0			556.8	0.93
325	4.5	do	0.8	1.07
0			132.2	1.20
400	4.5	do	0.0	1.14
0			156.4	1.26

a After one thousand galls had been counted each plant was discarded.

pounds per acre, respectively, and 2 replications of each rate. Injection depth was 6 inches. The treated area was 80 square feet per replicate. The treated soil was not covered. Lettuce transplants, free of Heterodera marioni, were set out in treated and control areas on March 25. On or around April 20 it was noted that plants growing in treated soil were smaller and darker green than adjacent plants in untreated soil; the higher the rate of application, the greater the injury. Later, the lettuce recovered except where application rate was 375 pounds per acre. Due to a misunderstanding, the grower cut all marketable heads of lettuce from treated and untreated areas on May 22. No weights were taken by him. Two days later, the writer counted the number of heads that had been harvested and found that 63 per cent were removed from untreated plots, and 70, 65, and 30 per cent from plots in which the soil had been treated with D-D mixture at 210, 290, and 375 pounds per acre respectively.

The root systems of 6 plants taken at random from each replicate were carefully removed, the soil was washed away, and the galls were counted. Plants grown in untreated soil averaged 660 galls per plant, while those grown in treated soil, regardless of the rate of application, averaged 3 galls per plant. Control of the root-knot nematode was as good at 210 pounds per acre as at the higher rates.

In Experiment No. 7, D-D mixture was injected as in Experiment No. 6, but at 275 and 550 pounds per acre, respectively, into Norfolk loamy sand, medium to well drained, organic matter fairly high, moisture-holding capacity 18–20 per cent, pH 6.0–6.2. This soil was known to be infested with Heterodera marioni, and was shown subsequently to be infested also with the fungus, Pythium aphanidermatum. The soil was treated on April 18, 1944, there being 2 replications of each rate per acre. Each replication consisted of 360 square feet. Adjacent untreated areas served as checks. On June 27, snap beans were planted, 4 rows in each treated and untreated area. On July 27, 40 plants, taken at random, were removed from treated and untreated areas with root systems intact, the soil was washed away, and the nematode galls were counted. One hundred and ten galls (mean) were found on the roots of plants grown in untreated soil, while no galls could be found on the roots of plants grown in the treated soil. Once again D-D mixture was an effective nematocide against Heterodera marioni.

Studies on Fungi

From repeated greenhouse tests, during the winter of 1943-44, in Woodstown silt loam infested with a Rhizoctonia-Fusarium complex capable of causing pre-emergence and post-emergence damping-off of spinach and preemergence damping-off (seed decay) of peas (Pisum sativum), it has been found that D-D mixture at rates up to 1,000 pounds per acre, trichlorobutanes at 250 pounds per acre, and monochlorobutenes at 450 pounds per acre, have very little value in the eradication of these fungi from an infested soil. Simultaneously, in the same soils, Arasan (2 per cent by weight) on spinach, and Spergon (2 per cent by weight) on peas, gave good to excellent control of damping-off. Soil treated with D-D mixture at 1,000 pounds per acre gave the first indication of reducing damage by damping-off fungi when 18 per cent more plants emerged from soil treated with the D-D mixture than from the untreated check soil, but Arasan used simultaneously reduced the damping-off from approximately 60 per cent in the check to less than 10 per cent when the seed treatment was used. Seed treatment is faster and easier, and consistently more effective. In these greenhouse tests the soil moisture was maintained at its optimum, and the pH ranged from 6.0 to 6.5. Soil temperatures 4 inches deep ranged from a minimum of 60-66° F. to a maximum of 61–78° F. Little phytocidal action by the D-D mixture was noted on germinating spinach planted 6 days after soil treatment, and none on spinach planted 14 days after soil treatment. There was some evidence of injury to the peas planted 10, 12, 15, and 22 days after soil treatment. Interestingly enough, the injury was not observed on peas planted 24 hours or 3 days after treatment.

Pythium aphanidermatum was found in the soil used in Experiment No. 7. In this test, designed to determine the value of D-D mixture as a nematocide at 275 and 550 pounds per acre, the fungus caused a 10 per cent post-emergence damping-off of the beans when the plants were $2\frac{1}{2}$ to 3 weeks old. One month after planting, counts of surviving plants were made in areas treated with D-D mixture or comparable untreated areas. Assuming the rate of seeding to be the same in all rows, and there seemed to be no reason for not believing this to be so, it was found that a mean of 95 plants were present in 30 feet of treated row, and 97 plants were present in a similar length of untreated row. Though there exists the possibility of recontamination with Pythium aphanidermatum from outside sources, it would seem from this simple test that D-D mixture at 550 pounds per acre did not inhibit the action of the fungus.

TABLE 4.—Mean number of tomato transplants, variety Marglobe, diseased with Fusarium wilt, growing in infested soil treated with D-D mixture before planting or left untreated. Sixty transplants set out per treatment and in checks

Treatment (pounds of D-D mixture		s surviving splanting	Plants	with wilt
per acre)	Number	Per cent	Number	Per cent
0 190 250 385 500 1000	29 26 27 27 30 34	48.3 43.3 45.0 45.0 50.0 56.6	23 22 19 20 22 24	79.3 84.6 70.3 74.0 73.3 70.5

Experiment No. 8 was designed to still further test the action of D-D mixture against soil fungi. A loamy sand, of low organic matter content and good drainage, 15-20 per cent moisture content, pH 5.5, which had just supported a crop of tomatoes almost 100 per cent diseased with Fusarium oxysporum f. lycopersici, was treated with D-D mixture at 190, 250, 385, 500, and 1,000 pounds per acre on July 10, 1944. The chemical was injected in holes 6 inches deep, at points 12 inches apart in staggered rows. There were 6 replications of each rate of application and, in addition, 6 check plots; each replicate was approximately 36 square feet. Two weeks after treatment of the soil, 10 tomato plants of Marglobe variety, free from root-knot, were set out in each replicate. Unusually hot weather caused a rather high plant mortality, but about 50 per cent survived; the plants' demise was not thought to be due to toxicity of D-D mixture. On October 15, stems of all plants were split and examined macroscopically for the discoloration indicative of infection with Fusarium. Enough isolations were made to check the belief that this discoloration was due to Fusarium oxysporum f. lycopersici. Findings (Table 4) show that D-D mixture, at rates up to and including 1,000 pounds per acre, applied to soil containing undecayed roots of tomato

plants that had died from Fusarium wilt, does not reduce the population of the pathogen as measured by the methods of the test. Taylor (6) found that chloropierin, at 200 pounds per acre, did not control *Heterodera marioni* when the soil contained fresh infected roots, and gave only fair control at 300 pounds per acre. However, good control was obtained at 200 pounds per acre applied after the nematode-infected roots had disintegrated.

DISCUSSION AND CONCLUSIONS

In the experiments reported here, D-D mixture, at 150 pounds per acre, has been an effective nematocide against the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. As a fungicide, D-D mixture has given disappointing results, for at rates as high as 1,000 pounds per acre it has failed to control damping-off of spinach and pea seed, caused by *Rhizoctonia sp.* (probably *R. solani*) and by *Fusarium sp.* (probably *F. martii*). The two additional soil fungi, *Pythium aphanidermatum* (Edson) Fitzpatrick and *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen, were not measurably affected by the presence of D-D mixture in the soil at rates of 550 and 1,000 pounds per acre, respectively. Seed treatment was more effective, and easier to use, in the control of the damping-off organisms.

Two other materials similar in chemical nature, namely monochlorobutenes and trichlorobutanes, had no fungicidal action at rates up to and including 450 pounds per acre, and no nematocidal action at 150 pounds per acre. Trichlorobutanes had decided phytocidal action, D-D mixture less, and monocholorobutenes none at all in these tests.

D-D mixture may be applied to cold soil (38-40° F.) and its effectiveness as a nematocide does not seem to be impaired. However, at low temperatures and relatively high dosages per acre (375 pounds) a toxic residue may remain in the soil, and lettuce transplants have been injured in these studies.

The writer has found D-D mixture easy to use and he has suffered no discomfort from its use. Spilled on the skin of the hands, and immediately wiped off, but not always washed off with soap and water as recommended by its manufacturers until some hours later, D-D mixture has not burned the skin. Its fumes are not pleasant to breathe, but if not inhaled unnecessarily they occasion no great precautions in its use. It is best to work on the windward side of the container, but D-D mixture has been used on many occasions in a closed greenhouse where it caused no ill effects to humans or to plants such as tomato, spinach, and cabbage growing within range of the fumes liberated from the material. Monochlorobutenes is likewise not unpleasant to use; trichlorobutanes is a dark, heavy, sticky liquid, which is difficult to clean from glassware, and probably also from metallic injection machines. Chloropicrin fumes are unpleasant and may damage growing plants near treated soil in closed places such as greenhouses.

SUMMARY

D-D mixture has been found to be an effective nematocide against *Heterodera marioni* (Cornu) Goodey at rates as low as 150 pounds per acre.

No covering of the soil, other than a possible wetting of the surface, seems necessary for good retention of fumes of the chemical. In limited comparisons, in which bush, snap, and Lima beans, tomatoes, potatoes, and celery were used, D-D mixture was the equal of chloropicrin as a nematocide. Two materials chemically similar to D-D mixture, namely monochlorobutenes and trichlorobutanes seem to possess no nematocidal values at 150 pounds per acre.

D-D mixture seems to possess little value as a fungicide in soil disinfestation studies with the damping-off fungi Rhizoctonia sp. (probably R. solani) and Fusarium sp. (probably F. martii), and with Pythium aphanidermatum (Edson) Fitzpatrick, and Fusarium oxysporum f. lycopersici (Sacc.) Snyder and Hansen. Dosages as high as 550 pounds per acre were not fungicidal to Pythium, and dosages as high as 1,000 pounds per acre did not control the other fungi.

D-D mixture has a slight phytocidal action if plants are set out in treated soil too soon after the treatment. At 150 pounds per acre, no injury has been found if 2 weeks elapse between treatment and time of planting. In cold soils, the time interval for safety may be 3 weeks or longer; limited studies indicate that the time interval varies with the dosage and also with the particular plant used. Lettuce has been injured when celery was not damaged.

D-D mixture may be applied to cold soil (30-40° F.) and its effectiveness as a nematocide does not seem to be impaired.

VIRGINIA TRUCK EXPERIMENT STATION, NORFOLK, VIRGINIA.

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INHERITANCE OF RESISTANCE TO BARLEY STRIPE^{1,2}

D. C. ARNY

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INTRODUCTION

Barley stripe, caused by Helminthosporium gramineum Rabh., occurs in nearly all countries where barley is extensively grown, but is of importance only in the more cool and humid regions (6, 16).3 In the United States infections have been usually less than 10 per cent, while reductions in yield of 5 per cent were fairly common.4,5 Stripe is an important disease in the winter barley areas and the West Coast, especially California.^{6, 7} In the spring barley areas of the upper Mississippi Valley the widespread use of Wisconsin Barbless, a somewhat resistant variety, has reduced the prevalence of the disease in recent years.

The cycle of infection for barley stripe and pathogenesis in relation to disease development have been well worked out. The earlier work has been reviewed by Leukel et al. (6), and more recently Stelzner (14) has reported further on the host-fungus relations. Conidia are produced in abundance on leaves, leaf sheaths, and culms of infected plants at about the time normal healthy plants are flowering, and are carried by air currents to florets of healthy plants. Under favorable conditions the conidia germinate and as the kernel develops the mycelium becomes established on and in the pericarp and aleurone layers, but not in the embryo. The following season when the kernels germinate the mycelium penetrates the young seedling, becomes systemic, and eventually invades all parts of the plant. Infected plants are more or less reduced in size, depending on the variety, and seldom produce plump germinable kernels.

Stripe can be controlled by seed treatment with the organic mercury dusts or similar surface disinfectants. However, it has been found [reviewed by Shands and Arny (12) after severe natural infection and artificial inoculation that a number of barley varieties are more or less resistant.

Artificial inoculation trials were started at Wisconsin, and were continued in connection with the barley breeding program. Since 1935 a large

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Dickson for generous aid and counsel during the investigations and preparation of the manuscript. The figures were prepared by Eugene Herrling.

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number of varieties and selections have been severely tested. With the cultures of the fungus used, some varieties have remained highly resistant while others have shown various degrees of resistance and susceptibility (12).

The inheritance of resistance to stripe was studied by Isenbeck (5).8

His type crosses and results may be summarized as follows:

Immune \times immune—62 lines showed no infection in the F₃ generation. Immune \times susceptible—among 88 lines, 82 were stripe-free in the F₃ generation, 6 had low infection. Resistance was apparently dominant.

Resistant \times resistant—some F_3 lines were more susceptible than either parent.

Susceptible \times resistant—dominance of resistance was again indicated. Susceptible \times susceptible—the majority of the F_s lines were susceptible, but some were more resistant than either parent.

Both wet and dry inoculations were used by Isenbeck. Only a single test was made on each line, and the reactions of the parents under comparable conditions were not indicated. A few lines had as much as 60 per cent infection. The large number of lines in all crosses which had no infection might be ascribed partly to the inadequacies of his inoculation techniques. In his results there were no sharply defined divisions between slight and high susceptibility, but rather continuous variation. He concluded that resistance was a heritable character, and that resistance was dominant in the varieties used. It was not possible to make a factorial analysis, although the absence of sharp divisions for infection groups suggested that several factors were involved. In one cross, susceptible × resistant, he used a "spore-kernel" inoculation method to test the lines, and found that the results agreed with those obtained by floral inoculation, but infection was more certain with the "spore-kernel" method.

The work of Shands et al. (13) indicated that factors for stripe reaction were inherited, but gave no suggestions as to the exact manner of their inheritance. From crosses involving resistant and susceptible parents, selections with various degrees of resistance and succeptibility were obtained. Backcrosses of resistant lines to the susceptible parent gave increased susceptibility.

Since resistance to stripe is important in the barley breeding programs in Wisconsin and other barley producing States, it seemed desirable to know more definitely the inheritance of the reaction to the disease. Attempts were made to determine how the resistance of certain varieties was inherited, and which chromosomes carried the factors for resistance.

MATERIALS AND METHODS

Inoculation Technique

The method of inoculation used in this work has been described (1, 12). It has given fairly consistent infection in susceptible varieties. The method s Also reported by Roemer et al. (11).

has been tested at various stages to determine what conditions were optimum for infection, and where the schedule could be changed when necessary without seriously affecting the results. In general, inoculum 2 to 4 days old has been best, although that 12 days old has given good results. The amount of infection was increased as the incubation period was lengthened, but beyond 4 days the increased amount of infection was more than offset by a decrease in the total number of plants developing beyond the early seedling stage. Seed of rye and barley used as the substrate for growth of the inoculum gave no better results than wheat. A higher water content in the wheat medium has given some increase in infection, but again this was offset by a decrease in the total number of plants surviving.

Source of Causal Organism

The culture of fungus used in all the present experiments has been designated as C-1. This culture originated from a single hyphal tip isolated from a Wisconsin collection in 1932 (12). Since then it has been carried in artificial culture by mass transfers. It has maintained a high degree of pathogenicity on Oderbrucker, and other barley varieties have reacted to it in essentially the same manner from year to year. There is very little chance that natural infection has affected the results, since practically no naturally infected plants have appeared on the Hill Farms during the time these experiments were carried out.

Parental Varieties

The stripe reactions of the varieties and selections used in the inheritance studies are in table 1. The reactions are given as averages only, but indications of the variability between tests are presented in figures 1, 2, 4, 5, and 6. Varieties in which there have been no stripe-infected plants, such as Persicum and Brachytic, have been classified as highly resistant. Varieties with infections from 2 to 15 per cent, such as the Lion selections, have been considered resistant. Oderbrucker, Colsess IV, and Iris have been highly susceptible under the conditions of these tests. In the results and discussion that follow these terms will be used in the sense indicated.

Lion is a black barley which has been used as the smooth-awned parent for several varieties grown commercially in the United States. The two selections were alike in all respects, except that Lion 28 has seemed to be slightly more susceptible to stripe than Lion 36. Persicum is a smooth-awned, 2-rowed variety that came from Russia. Brachytic, a selection with much shortened internodes, originated as a mutant in normal Himalaya (8). Oderbrucker is a rough-awned variety which was the standard malting barley in Wisconsin for many years. Robertson (9) developed the stock Colsess IV, which carries the lethal seedling character, xantha, in a heterozygous condition, but in the work reported here only the homozygous green lines of this stock were used. Iris is a rough-awned, naked-kerneled variety of no commercial importance.

Beside their stripe reactions these varieties had other differentiating morphological characters known to mark particular linkage groups. Thus, crosses have been studied for these characters as well as for stripe reaction. Classifications for these characters were made on F_2 plants and verified in noninoculated F_3 families.

TABLE 1.—Reactions of parental varieties when inoculated artificially with Helminthosporium gramineum Rabh. (Culture C-1) in field and greenhouse trials at Madison, Wisconsin from 1939 to 1944a

Voniska	01 T 3 T .	Location of		Stri	Stripe infected plants					
Variety C.I. No.b		trials	Trials	Low	High	Weighted average				
			No.	Pct.	Pct.	Pct.				
Lion 28	923 sel.	Greenhouse Field	$\begin{array}{c} 62 \\ 16 \end{array}$	6 5	$^{48}_{16}$	15 9				
Lion 36	923 sel.	Greenhouse Field	$\begin{array}{c} 56 \\ 32 \end{array}$	2 5	10 16	6 8				
Persicum	6531	Greenhouse Field	$\frac{104}{21}$	0	0	0				
Brachytic	6572	Greenhouse Field	54 76	0	0	0				
Colsess IV	5979	Greenhouse Field	32 38	56 67	86 82	73 77				
Oderbrucker	4666	Greenhouse Field	$\frac{212}{107}$	79 69	91 91	84 78				
Iris	998	Greenhouse Field	23 5	80 89	93 95	81 91				

^a These reactions are based on tests not included in a previous report on stripe reaction of varieties (12).

b C.I. denotes accession number of the Division of Cereal Crops and Diseases (Formerly Office of Cereal Investigations), Bureau of Plant Industry, U. S. Department of Agriculture.

^c Low and high figures are yearly averages. The weighted average is the sum of the yearly average multiplied by the number of tests in each year, divided by the total number of tests.

Method of Testing Segregates

Hybrid material was tested in the F_3 and to a limited extent in the F_1 , F_2 , and F_4 generations. Because inoculation did not eliminate all plants of a susceptible variety such as Oderbrucker, tests of F_2 plants could not be expected to give a completely reliable indication of their reactions. Inoculation of the F_3 lines, where a large population and several trials could be used, have therefore, been considered to give a more nearly true picture of the reaction of F_2 plants. Twenty-five or thirty kernels per line were used in each inoculation, and each progeny was tested more than once in attempting to eliminate possible escapes or sampling errors. Oderbrucker and the other parental varieties concerned in any particular trial were used as checks, and included once for every 17 or 18 hybrid lines. A number of tests were made of the F_1 and random samples of the F_2 material. The F_3 lines were taken at random from noninoculated populations except in one instance, and each line was tested at least twice. In general, all the F_3 lines

of a cross were tested at once, but because of limited facilities it was not possible to test each line more than once at a given time. Thus the trials were not strictly replications, and comparisons between them could be made only on the basis of the percentage infection of Oderbrucker and the susceptible parent in the respective trials. F_4 lines were made up by bulking the plants from noninoculated rows of F_3 lines which had been highly susceptible or resistant in other tests. Duplicate inoculations were made on F_4 lines.

The stripe disease has been relatively easy to classify, as in most cases the symptoms on stripe-infected plants were obvious, and plants were either healthy or distinctly stripe-infected. Occasionally, questionable plants appeared, but not frequently enough to have any appreciable effect on the results.

INHERITANCE OF RESISTANCE

$Lion \times Oderbrucker$

As shown in table 1 Lion 36 has been resistant under conditions which produced high amounts of infection in Oderbrucker. The reactions of the F_1 and F_2 generations of Lion $36 \times \text{Oderbrucker}$, with the checks of parent varieties, are shown in figure 1. On the basis of the average infection both generations were susceptible. No significant differences appeared between progenies from reciprocal crosses. Lion 28 has appeared to be somewhat more susceptible than Lion 36. In the F_1 of Lion $28 \times \text{Oderbrucker}$ the amount of infection was slightly less than in Lion $36 \times \text{Oderbrucker}$

PARENT OR CRO		REPLI- CATIONS	PLANTS		PERG	MBER OI CENTAGE CLAS	E INFE		N		AVERAGE INFECTION
				Ϋ.	1,5	3.5	5.5	,	7,5	9,5	
		NO.	NO.								PER CENT
ODERBRUCKER P		47	996								8.5
LION 36 P		27	510			EUSN.					7
LION 28 P		. 19	356	1315		-					20
LION 36 X											
ODERBRUCKER	F ₁	6	. 81						-		7.8
RECIPROCAL	Fi	9	153			Marata .		-	******		57
LION 36 X											
ODERBRUCKER	F ₂	4	79					_			6 6
RECIPROCAL	F ₂	8	94					(A00,00			66
LION 28 X											
ODERBRUCKER	Fi	9	143				. Minne	M · manual	-		63
RECIPROCAL	Fi	5	82			10000		-			46
LION 28 X	•										•
ODERBRUCKER	F ₂	4 4	1154								42
ODERBRUCKER *	Ρ	1	38								97
LION C	P	1	109		•						3
	F ₂		117							- 1	81

Fig. 1. Lion × Oderbrucker. Distribution of tests of parents and F₁ and F₂ populations in percentage classes according to the stripe infection when artificially inoculated. ^a Parents and F₁ and F₂ populations were placed in infection classes on the basis of individual tests, while F₃ lines and backcross lines were placed on the basis of the averages of 2, 3, or 4 trials as indicated. The height of the column in each class is proportional to the number of tests or lines which fell in that class.

b 10.1 to 20.0 per cent inclusive. c Unpublished data from H. L. Shands, 1932. brucker, and in the F_2 the amount was distinctly less. This was due in part to a lesser infection in the trial in which the major portion of the tests of the F_2 of Lion 28×0 derbrucker was run. In an earlier test it was found that the F_2 of Oderbrucker × Leiorrynchum (Lion) was very susceptible, as indicated by the data at the bottom of figure 1. From the reactions of the F_1 and F_2 generations it appeared that the susceptibility of Oderbrucker was at least partially dominant.

One hundred sixty-two F_3 lines taken at random from a noninoculated F_2 population were tested 4 times each for their stripe reaction. An analysis of variance of the F_3 data transformed to \sin^2 (because the data were expressed as percentages) indicated that there was some differential reaction

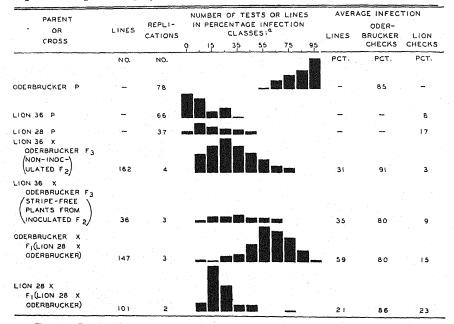


Fig. 2. Lion × Oderbrucker. Distribution of parents, F₂ lines, and backcross lines in percentage classes according to the stripe infection when artificially inoculated.
^a See footnote for Fig. 1.

in lines between replications, but it did not appear to be serious. The lines were placed arbitrarily in 10 per cent reaction classes on the basis of the average infection for 4 replications. When the transformation was used the standard error within these classes was uniform over the whole range of infection percentages, and the minimum significant differences were only slightly larger than the classes. Similar statistical treatments were given the crosses to be discussed subsequently, and the results were almost identical.

The distribution of parental checks and F_3 lines (from noninoculated F_2) is shown in figure 2. The distribution of the lines was fairly normal, though skewed toward the resistant side, with the mode in the 25 per cent

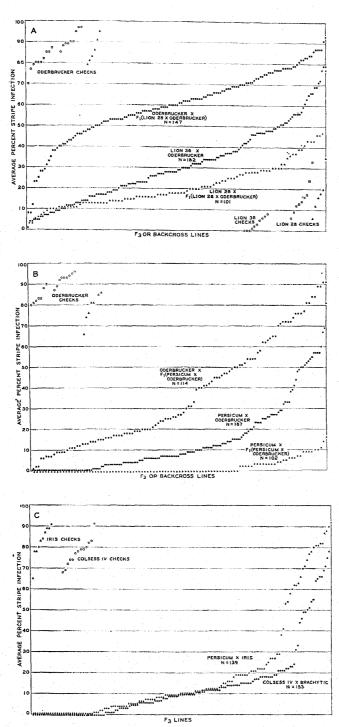


Fig. 3. Distributions of parents, F_3 lines, and backcross lines in order of increasing average stripe infection. Each solid black mark represents a line.

class and the mean in the 35 per cent class of infection. As would be expected from the fact that Lion 36 was not completely resistant there were no F₃ lines in the 0 per cent class. There were few lines in the susceptible end of the distribution, and it was doubtful whether any lines were as susceptible as Oderbrucker. Although the inoculation treatment was rather severe there did not appear to be any marked selective mortality before emergence, since the number of plants per line was nearly as high in the susceptible classes as in the resistant. This is indicated by the following:

Percentage infection classes	5	15	25	35	45	55	65	75
Average number of plants per line (100 kernels planted)	66	68	67	65	64	64	63	69

The distribution of the F₃ lines in order of increasing average stripe infection is shown in figure 3, A. The curve formed showed a continuous variation, without discrete classes of resistance or susceptibility.

Thirty-six lines obtained from F_2 plants which remained stripe-free under a severe inoculation of an F_2 population were tested for stripe reaction. About 65 per cent of the F_2 plants had been eliminated by stripe infection. The distribution of the remaining lines, based on the average of 3 tests each, is given in figure 2 under the designation (Inoculated F_2). The distribution appeared to be very similar to that of the lines from the noninoculated F_2 . The data indicated that the F_2 test did little to select for resistant plants.

Part of the lines from the noninoculated F_2 population were tested further in the F_4 . These lines were chosen because they were either high or low in amount of infection in the F_3 tests. The F_4 lines were made up by bulking plants from noninoculated F_3 rows. The data are in table 2. The infection averages for the 2 generations agreed very well. In these tests it appeared that lines 21, 84, and 106 were as susceptible as Oderbrucker, and 13 lines were as resistant as Lion 36, if 15 per cent were considered the upper limit for resistance.

F_1 (Lion 28 × Oderbrucker) × Oderbrucker

The backeross plants were grown without inoculation, and the lines obtained tested for stripe reaction. Results for reciprocals were combined because of their similarity. The distribution of the lines, as given in figure 2, showed a definite shift toward susceptibility when compared to the simple cross. The average infection in the F₃ lines was increased by 28 per cent by the introduction of another complement of the susceptible genotype. The distribution of the backeross lines in order of increasing average infection is shown in figure 3, A. The curve showed considerably higher infection than that for the simple cross, and there appeared to be no obvious breaks in continuity.

F_1 (Lion 28 × Oderbrucker) × Lion 28

The distribution of the lines of this backcross tested for stripe reaction also appear in figure 2. The results for reciprocals were combined.

Eighty-five per cent of the lines seemed to be as resistant as the Lion 28 The 3 lines in the 0 per cent class showed some stripe on further The resistance of Lion 28 appeared to be dominant in this cross. The distribution of the lines in order of increasing average infection is

TABLE 2.—Comparison of percentage stripe infection of high and low F3 lines with percentage infection in corresponding F4 lines made up by bulking uninoculated F3 rows

number Averagea Averageb number Averagea Averagea <t< th=""><th>Lic</th><th>$n \times Oderbruch$</th><th>ker</th><th>Persicun</th><th>$1 \times Oderbrue$</th><th>ker</th></t<>	Lic	$n \times Oderbruch$	ker	Persicun	$1 \times Oderbrue$	ker
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$					F_{a} Average	F ₄ Average
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		Pct.	Pct.		Pct.	Pct.
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	65	1	9	27	81	64
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$						36
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		4	32	97	69	40
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$				34	60	28
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$				72	57	22
167 5 10 110 57 1 50 6 14 28 55 2 93 6 6 7 54 2 14 7 17 166 53 1 83 7 15 57 52 3 186 7 10 43 51 2 18 8 7 Ave. 51 2 43 8 19 190 8 27 Oderbrucker 93 7 90 9 9 9 Persicum 0 0 105 9 12 Brachytic × Oderbrucker 93 7 98 10 6 Line F ₃ Ave. 7 14 number Averaged Ave 106 79 71 Pct. P 120 77 48 15 58 5 124				100		18
50 6 14 28 55 2 93 6 6 6 7 54 2 14 7 17 166 53 1 83 7 15 57 52 3 186 7 10 43 51 2 18 8 7 Ave. 51 2 43 8 19 19 Ave. 51 2 43 8 19 Oderbrucker 93 7 90 9 9 9 Persicum 0 105 9 12 Brachytic × Oderbrucker 98 10 6 Line F3 Ave. 7 14 number Averaged Ave 106 79 71 Pet. P 150 79 62 208 65 4 12 77 48 15 58<				110		17
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$				28	55	27
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$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$				166		13
186 7 10 43 51 2 18 8 7 Ave. 51 2 43 8 19 Oderbrucker 93 7 190 8 27 Oderbrucker 93 7 90 9 9 9 Persicum 0 105 9 12 Brachytic × Oderbrucker 98 98 10 6 Line F3 Ave. 7 14 number Averaged Ave 106 79 71 Pet. P 150 79 62 208 65 4 12 77 48 15 58 3 124 72 62 26 52 3 44 71 48 29 41 2 21 69 71 47 47 39 92 69 54 84 38				57		35
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12 77 48 15 58 3 124 72 62 26 52 3 44 71 48 29 41 2 21 69 71 47 39 92 69 54 84 38 4 84 66 76 65 37 3 158 66 63 136 36 3 77 65 66 48 35 4 69 63 65 126 35 3 15 61 33 97 32 3 Ave. 70 60 Ave. 43	106	79	71		Pct.	Pct.
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44 71 48 29 41 2 21 69 71 47 39 92 69 54 84 38 4 84 66 76 65 37 3 158 66 63 136 36 3 77 65 66 48 35 4 69 63 65 126 35 3 15 61 33 97 32 4 Ave. 70 60 Ave. 43 3						37
21 69 71 47 39 92 69 54 84 38 4 84 66 76 65 37 5 158 66 63 136 36 3 77 65 66 48 35 6 69 63 65 126 35 3 15 61 33 97 32 6 Ave. 70 60 Ave. 43						26
92 69 54 84 38 4 84 66 76 65 37 5 158 66 63 136 36 36 77 65 66 48 35 4 69 63 65 126 35 35 15 61 33 97 32 4 Ave. 70 60 Ave. 43						6
84 66 76 65 37 3 158 66 63 136 36 3 77 65 66 48 35 4 69 63 65 126 35 3 15 61 33 97 32 3 Ave. 70 60 Ave. 43						45
158 66 63 136 36 36 77 65 66 48 35 4 69 63 65 126 35 3 15 61 33 97 32 4 Ave. 70 60 Ave. 43 3						33
77 65 66 48 35 69 69 63 65 126 35 61 15 61 33 97 32 62 Ave. 70 60 Ave. 43 43						39
69 63 65 126 35 15 61 33 97 32 Ave. 70 60 Ave. 43						46
15 61 33 97 32 4 Ave 70 60 Ave 43						30
Ave						58
						37
	Odebrucker		75	Oderbrucker	. 74	60
Odebrucker 91 75 Oderbrucker 74 Lion 36 3 7 Brachytic 0						0

^a Average of 4 trials, 2 in field and 2 in greenhouse.

shown graphically in figure 3, A. Again there were no obvious breaks in the curve, and infection percentages were lower than for the simple cross.

b Average of 2 trials in greenhouse. c Average of 3 trials.

d Average of 4 trials.

e Average of 2 trials.

The data for the several generations of Lion \times Oderbrucker were somewhat contradictory. The amount of infection in the F_1 and F_2 indicated that susceptibility was at least partially dominant; however, the distribution of the F_3 lines indicated that resistance was partially dominant. Tests of selected F_4 lines agreed with the F_3 findings, suggesting that the F_3 classifications were essentially correct, and that the F_3 lines with high and low amounts of infection were relatively pure for stripe reaction. The results with F_3 lines from an inoculated F_2 population suggested that the inoculation of F_2 material had no selective effect towards resistance. If the tests of the F_3 were considered the most reliable, then the skewness of the distribution of lines would indicate that resistance was partially dominant, and the normality of the distribution would suggest that several factors were involved in conditioning stripe reaction. The backcross of the F_1 to Oderbrucker, in which 10 per cent of the lines fell in or above the 85 per cent infection class, suggested that a 3-factor difference was involved.

PARENT OR CROSS REPLI- AND GENERATION CATIONS		PLANTS		PE		AVERAGE INFECTION			
			o	15	35	55	. 75	95	
	NO.	NO.		4					PER CENT
ODERBRUCKER P	47	996							8.5
PERSICUM P	13	289		_					0
PERSICUM X ODERBRUCKER	F ₁ 22	338		322					
PERSICUM X ODERBRUCKER	F ₂ 26	557							6

Fig. 4. Persicum \times Oderbrucker. Distribution of parents and F_1 and F_2 populations in percentage infection classes according to the stripe infection when artificially inoculated.

The backcross of the F₁ to Lion seemed to substantiate this possibility by showing 85 per cent of the lines in the 25 per cent infection class or below. The resistance of Lion was apparently dominant in these backcross lines. The ease with which parental types were regained in backcrosses suggested that the inheritance was not complex.

$Persicum \times Oderbrucker$

Persicum has been highly resistant in all inoculation tests made with culture C-1. The reactions of the F_1 and F_2 generations of the cross Persicum × Oderbrucker are given in figure 4. The results for reciprocals were combined. Most of the F_1 plants were resistant when either variety was used as the female parent. The F_2 generation had a slightly higher number of stripe-infected plants. The resistance of Persicum appeared to be dominant.

The distribution of the F₃ lines, based on the average infection, is shown in figure 5. A large proportion of the lines were highly resistant, with 20 per cent of them in the 0 per cent infection class. No lines appeared to be as susceptible as Oderbrucker, although one line in the 85 per cent class

approached it. The distribution of the F_3 lines in order of increasing average infection appears in the middle curve of figure 3, B. The curve formed suggested no definite divisions except the one susceptible line and the highly resistant class. No differential mortality appeared in the higher infection classes as the average number of plants per line in the higher classes was nearly the same as in the lower classes, as shown in the following:

Percentage infection classes	0	5	15	25	35	45	55	65 75	85
Average number of plants per line (75 kernels planted)	68	70	68	68	66	64	63	73	44

There was a slight downward trend from the 5 per cent class to the 55 per cent class. The 65 and 85 per cent classes contained only 2 lines and 1 line, respectively.

PARENT				NUMBER OF TESTS OR LINES AVERAGE	NFECTION
OR CROSS		LINES	REPLI- CATIONS	CLASSES:" LINES BRUG	ER- PERS- CKER 1CUM CKS CHECKS
		NO.	NO.	PCT. F	CT. PCT.
ODERBRUCKER	P	_	62		85 -
PERSICUM	P	-	62	-	
PERSICUM X ODERBRUCKER	l Fa	167	3	62	93 0
ODERBRUCKER > F ₁ (PERSICUM > ODERBRUCKE	(81	3		84 0
RECIPROCAL		33	. з	25	84 0
PERSICUM X					
F _I (PERSICUM : ODERBRUCKE		102	2	71	78 0

Fig. 5. Persicum \times Oderbrucker. Distribution of parents, F_3 lines, and backcross lines in percentage infection classes according to the stripe infection when artificially inoculated.

Lines that were highly resistant or relatively susceptible in the F_3 were tested again in the F_4 as a check on the F_3 classification. These F_4 lines were from bulked, noninoculated F_3 rows. The averages for the 2 generations of these lines are in table 2. The inoculation tests for the F_4 lines were less severe than for the F_3 lines, as is shown by the lower average for the Oderbrucker checks. With the exception of lines 68 and 87 those lines which showed no infection in the F_3 also showed none in the F_4 . There were 17 lines with no stripe-infected plants in either the F_3 or the F_4 . Lines with high infection in the F_3 had some susceptibility in the F_4 , but only line 27 appeared to be as susceptible as Oderbrucker.

F_1 (Persicum × Oderbrucker) × Oderbrucker

 F_1 plants of Persicum × Oderbrucker were backcrossed to Oderbrucker, and the crossed seed grown to produce backcross lines. The F_1 plants were

[,] a See footnote for Fig. 1.

used both as male and female parent. The distribution of the backcross lines is shown in figure 5. The average infection in the lines in which the hybrid was the female was less than in those in which Oderbrucker was the female (21 per cent compared to 41 per cent). The distributions of the progenies from reciprocal crosses were essentially similar, however, and both showed a definite trend towards susceptibility when compared to the distribution for the simple cross. There were 18 lines in the 75 and 85 per cent classes which thus appeared to be as susceptible as the Oderbrucker checks. Since every backcross line had at least half of the Oderbrucker gene complement, and resistance was not completely dominant, no lines would be expected in the 0 per cent class. The distribution of the lines in order of increasing average infection is shown in the upper curve of figure 3, B. Breaks in the distribution were suggested at the 35 and 65 per cent levels.

F_1 (Persicum × Oderbrucker) × Persicum

Similar crosses were made with Persicum as the recurrent parent, and a limited number of lines tested for stripe reaction (Fig. 5). Seventy per cent of the lines appeared to be stripe-free, and the balance showed only a low percentage of infection. Resistance appeared to be partially dominant. The distribution of the lines in order of increasing average infection is shown in the lower curve of figure 3, B.

Based upon the amount of infection in the F_1 population of Persicum \times Oderbrucker it may be concluded that the factors responsible for resistance in Persicum were almost completely dominant over the factors for susceptibility in Oderbrucker. This dominance was evident also in the results of the studies of the F_2 , F_3 , and backcrosses. In the several tests of F_2 populations there was a total of 525 healthy plants and 32 stripe-infected plants. This very closely approximates a 15 to 1 or dihybrid ratio, P lying between 0.95 and 0.50. This would suggest that Persicum differed from Oderbrucker in 2 factor pairs, either one of which gave resistance. This hypothesis does not take into account the escapes in the F_2 tests.

The behavior of the F_3 and backcross lines also suggests a factorial hypothesis for the difference in stripe reaction between Persicum and Oderbrucker. In the distribution of F_3 lines approximately 20 per cent of the lines were as resistant as Persicum, and only 1 line (less than 1 per cent) as susceptible as Oderbrucker. These 2 classes may be considered as homozygous resistant and susceptible respectively, although the resistant class likely contained some heterozygous lines. All the lines between these extremes were probably heterozygous in various degrees and there appeared to be no logical means of separating them further. Although 2 factors were suggested by the F_2 tests, the low number of susceptible lines in the F_3 made it seem likely that at least 1 additional factor was involved.

If it is assumed that Persicum has 3 independent, dominant factor pairs for resistance, and that Oderbrucker has the recessive allelomorphs for complete susceptibility, the F_2 plants of a cross between these 2 genotypes (AABBCC and aabbcc) might be grouped on the basis of their F_3 breeding behavior as follows: 10/64 would give no segregation in the F_3 for at least 2 dominant factor pairs and would be highly resistant in the F_3 , 53/64 in the F_3 would segregate or be homozygous recessive for at least 2 of the factor pairs, and 1/64 would be true-breeding recessive in the F_3 and completely susceptible.

This would give a ratio of 10 highly resistant: 53 intermediate: 1 susceptible. The comparison of the calculated with the observed ratio indicates that the agreement was satisfactory.

	Observed	Calculated	
Highly resistant	34	26.1	$\gamma^2 = 3.66$
Intermediate	132	138.3	P̃ lies between 0.2 and 0.1
Susceptible	1	2.6	

On the basis of this hypothesis it would be expected that one-eighth of the backcross lines, with Oderbrucker as the recurrent parent, would be susceptible. If the backcross lines in the 75 and 85 per cent infection classes were considered as susceptible, the agreement of the observed segregation with the expected was good.

	Observed	Calculated	
Intermediate	96	100	$\chi^2 = 1.30$
Susceptible	18	14	P̃ lies between 0.3 and 0.2

In the backcross with Persicum as the recurrent parent about 70 per cent of the lines were highly resistant. This suggested a 2-factor difference between the 2 parents. If this backcross were to fit the 3-factor hypothesis suggested above, 50 per cent of the lines should show some stripe-infected plants. This was obviously not the case, although further tests might bring closer agreement.

$Brachytic \times Oderbrucker$

Brachytic has been highly resistant in tests using culture C-1. In crosses with Oderbrucker the F_1 and F_2 generations reacted to stripe as shown in figure 6, A. The reactions were essentially the same as in the cross Persicum \times Oderbrucker.

Four stripe tests were made with each F_3 line of this cross. One of these was in the greenhouse and the others were in the field. Because of dry soil the field tests were poor and the infection very low. The distribution of the F_3 lines on the basis of the average of the 4 tests is given in figure 6, A. It was very similar to that for Persicum × Oderbrucker, except that the average infection for all lines was lower. The distribution of the lines in order of increasing average infection was also every similar and thus is not shown here. Again a few lines approached the susceptibility of Oderbrucker. This lack of susceptible lines did not seem to be the result

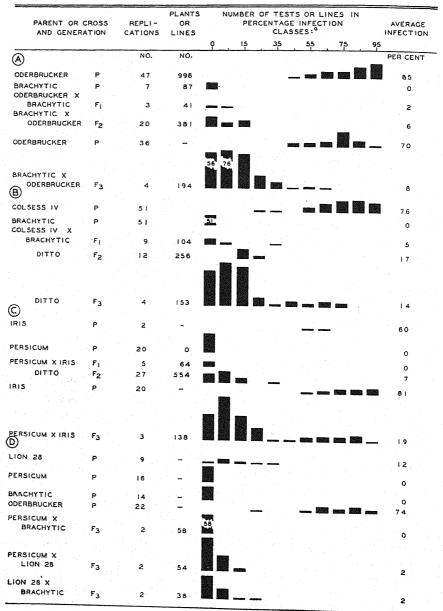


Fig. 6. Brachytic × Oderbrucker, Colsess IV × Brachytic, Persicum × Iris, Persicum × Brachytic, Persicum × Lion 28, and Lion 28 × Brachytic. Distribution of parents, F_1 and F_2 populations, and F_3 lines in percentage infection classes according to the stripe infection when artificially inoculated.

a See footnote for Fig. 1.

of differential mortality of stripe-infected plants before emergence as shown in the following:

Percentage infection classes	0	5	15	25	35	45	55	65
Average number of plants per line (120 kernels planted)	76	66	67	68	66	84	62	87

There were only 2 and 3 lines in the 55 and 65 per cent classes respectively.

Lines with no infection or with relatively high infection in the F_3 were tested again in the F_4 as bulks from noninoculated F_3 rows. The amount of infection in the Oderbrucker checks in these tests was low. A comparison of the F_3 and F_4 is in table 2. With the exception of lines 58, 114, 118, and 142, lines with no infection in the F_3 had none in the F_4 . These exceptions indicated that some escapes occurred in the F_3 tests. There were 19 lines with no stripe-infected plants in either the F_3 or the F_4 . Lines that were high in infection in the F_3 tests also had fairly high infection in the F_4 .

In general the several generations of the cross Brachytic × Oderbrucker have reacted to stripe inoculation in the same manner as did those of the cross Persicum × Oderbrucker. The presence of 2 dominant factor pairs in Brachytic was suggested by the F₂, as the agreement with the 15 to 1 ratio expected on this basis was satisfactory, P lying between 0.5 and 0.3. There were 362 healthy plants and 19 stripe-infected plants in the several F₂ tests. Because the F₃ tests were not severe, infection in the lines was generally low. Resistance appeared to be dominant over susceptibility, and apparently more than 1 factor difference was involved. The significance of the fact that slightly more than one-fourth of the F3 lines were highly resistant was problematical. Tests of certain F4 lines showed that the F3 classifications were essentially correct, and that some degree of homozygosity for stripe reaction had already been reached within the lines. The 3-factor hypothesis which seemed to fit the Persicum × Oderbrucker F₃ and backcross results, did not give satisfactory agreement for the progeny of Brachytic × Oderbrucker.

Colsess $IV \times Brachytic$

Colsess IV has been highly susceptible to the culture of the stripe fungus used in these tests. The results for Colsess IV × Brachytic are shown in figure 6, B. Inoculation of F_1 plants gave 8 per cent infection, indicating a partial dominance for the Brachytic resistance. In the F_2 generation 212 plants remained healthy and 44 became stripe-infected, a ratio of 4.8 to 1 or an average of 17 per cent infection. The distribution of the F_3 lines was distinctly different from the Lion × Oderbrucker or the Persicum × Oderbrucker type, because in Colsess IV × Brachytic there were relatively more susceptible lines. Twenty-five per cent of the lines had no infection, and 6 per cent had above 60 per cent. If the distribution is divided accordingly the segregation closely approximates that expected on the basis of a difference of 2 factor pairs, 1 major and 1 modifier.

ved Calculated	
$\frac{38.2}{105.2}$	$\chi^2 = 0.109$ P lies between 0.95 and 0.5

This division at the 60 per cent infection level was indicated by the distribution of the lines in order of increasing average infection as shown in figure 3, C. There was also a rather definite break at the 25 per cent level, but the significance of this was not evident.

$Persicum \times Iris$

Iris has been highly susceptible to the fungus culture used in these tests. Iris was crossed with Persicum, and the several generations tested for stripe reaction. The distributions are given in figure 6, C. Only a few plants were tested in the F_1 but they all remained healthy, indicating that resistance was dominant. In the F_2 there were 299 disease-free plants and 19 stripe infected. This closely approximates a 15 to 1 ratio ($\chi^2 = 0.05$; P lies between 0.95 and 0.50).

The distribution of the F_3 lines was very similar to that of the Colsess \times Brachytic F_3 progenies. There were 11 lines with 75 per cent or more infection. If these were considered susceptible then the observed and calculated ratios would be as follows:

	Observed	Calculated	
Highly resistant Intermediate Susceptible	100	33.6 92.4 8.4	$\chi^2 = 2.20$ P lies between 0.5 and 0.3

This again indicated a 2-factor difference with 1 major and 1 modifying factor. However, the break at the 75 per cent level might be questionable, as it was not definitely indicated on the curve in figure 3, C, formed by the distribution of the lines in order of increasing average infection. This curve is strikingly similar to the corresponding one for Colsess IV × Brachytic.

$Persicum \times Brachytic$

Both of the varieties in this cross have been highly resistant in tests with culture C-1. F_3 lines of the cross have been tested for their stripe reaction. Figure 6, D, shows that while fairly high amounts of infection occurred in the Oderbrucker checks, no stripe-infected plants appeared in any of the F_3 lines or in either of the parents. This lack of segregation would indicate that there were some factors for resistance common to both Persicum and Brachytic.

Persicum × Lion 28

The F_2 of Lion×Persicum gave very low infection on inoculation, with an average of 2.5 per cent in 8 tests involving 198 plants. F_3 lines have also been tested and their distribution is shown in figure 6, D. The average infection for the F_3 lines was very low, but stripe-infected plants

appeared in some lines. In none of the lines did the amount of infection exceed that which occurred in the Lion 28 checks. Because segregation occurred in the F_3 lines of this cross it may be concluded that there was either a quantitative or a qualitative difference in the factor or factors conditioning resistance in the 2 varieties.

Lion 28 × Brachytic

The distribution of the F_3 lines is shown in figure 6, D. This cross was very similar in its behavior to the Persicum × Lion 28 cross and the conclusions drawn were the same.

ASSOCIATION OF CHARACTERS

In addition to the studies of the stripe reactions of the progenies of the various crosses, observations were made concerning the inheritance of certain morphological characters. Classifications were based upon F_2 plants and verified in the F_3 lines, except in the backcrosses, where only the backcross plants were classified. Although the populations were relatively small, attempts were made to verify previous reports as to the mode of inheritance of the genes and their relationships to each other. A summary of linkage studies and a list of references on mode of inheritance has been given by Robertson, Wiebe, and Immer (10), and therefore most of the references will not be cited here. Tests for goodness of fit and independence were made by means of Chi-square. These tests are not reported in detail, but a P value of 0.95 to 0.05 was regarded as indicating satisfactory agreement. The principal object was to determine the relations of these genes to stripe reaction.

$Lion \times Oderbrucker$

Lion 36 has a black lemma and pericarp, smooth awns, long-haired rachillas, practically no hairs on the glumes, and glume awns 7 to 10 mm. long. In contrast Oderbrucker has a white lemma and pericarp, rough awns, short-haired rachillas, glumes with abundant hairs, and glume awns 16 to 25 mm. long.

Long-haired vs. short-haired rachilla and black vs. white lemma and pericarp have been reported to be conditioned by single independent factor pairs. The general classification of rough vs. smooth awns has been found previously to be conditioned by a single factor pair, with at least 1 other pair modifying the degree of smoothness. Neatby (7) has determined that in certain varieties glume length acts as a dihybrid in its inheritance. Hor (3) reported that the factor affecting rachilla hair length also affected the nature of pubescence of the glume in a similar way, while the extent of coverage of the glumes by hairs was governed by another factor pair. Daane (2) found the factors governing rough vs. smooth awns and long- vs. short-haired rachillas to be linked.

In the cross Lion 36 × Oderbrucker the inheritance of rachilla hair

length, glume awn length, lemma color, and rough vs. smooth awns were found to be governed by a single factor pair in each case. Glume pubescence was an unsatisfactory character to classify because all gradations from no hairs through various widths of bands of hairs to complete coverage occurred. At least 3 factor pairs were apparently involved in the inheritance of glume pubescence in this cross. The factors for rough awns and short-haired rachillas appeared to be linked, with a percentage recombination of 26 ± 2.9 when computed according to the method of Immer and Henderson (4). Hairy glumes appeared to be linked with both long glume awns and rough awns. Factors for other characters studied seemed to segregate independently of each other. By observation (Table 3) the stripe classes showed no definite association with any of these 5 characters, and independence was indicated by Chi-square tests in all instances.

Lemma color, rachilla hair length, and stripe reaction were studied in the backcross Oderbrucker \times F_1 (Lion $28 \times$ Oderbrucker) and its reciprocal. The reciprocals were considered separately, but there was no evidence of disagreement between them. It was found that the lemma color and rachilla hair length were each monogenic, and independent of each other and of the stripe classes. The average stripe reactions for the several genotypes are in table 3.

Glume awn length, rough vs. smooth awns, glume pubescence, and stripe reaction were studied in the backcross Lion $28 \times F_1$ (Lion $28 \times \text{Oderbrucker}$) and its reciprocal. Glume awn length and rough vs. smooth awns were monogenic and independent in their inheritance. Glume pubescence here appeared to be governed by 2 factors and hairy glumes were associated in some way with both long glume awns and rough awns. All 3 morphological characters were independent of stripe reaction. The independence of the characters and stripe reaction was also indicated by the infection averages for the various genotypes in table 3.

$Persicum \times Oderbrucker$

Persicum has a black lemma and pericarp, smooth awns, long-haired rachillas, short glume awns, and infertile lateral florets (2-rowed type). Oderbrucker has fertile lateral florets, as well as the characters described under the previous cross. Segregation for lemma color, rough vs. smooth awns, rachilla hair length, and glume awn length agreed satisfactorily with the assumptions of monogenic inheritance. Varieties with infertile lateral florets have been reported by a number of workers to differ in their genotype from those with fertile lateral florets by a single dominant factor pair. In the present study, the segregation for row number did not fit a 1:2:1 ratio because of an excess of non-2-rowed segregates. F₂ plants were taken at random to make up the F₃ lines, the only restriction being that there be sufficient kernels on each for the F₃ tests. This would tend to favor the non-2-rowed class at the expense of the 2-rowed and heterozygous classes because these latter produce relatively few kernels per plant.

When the remainder of the population from which the lines were taken was classified and the results combined with those for the F3 lines tested for

TABLE 3.—Number of lines and average infection for various genotypes and several crosses

Cross	Homozygous dominant			Heterozygous			Homozygous recessive		
	Geno- typea	Lines	Ave. stripe infec.	Geno- type ^a	Lines	Ave. stripe infec.	Geno- type ^a	Lines	Ave. stripe infec.
		No.	Pct.		No.	Pct.		No.	Pct.
Lion 36×	SS	45	33	Ss	85	31	SS	32	31
Oderbrucker	$\mathbf{E_{2}E_{2}}$	31	30	$\mathbf{E_2e_2}$	89	33	e_2e_2	42	29
	$^{ m BB}$	36	37	${f Bb}$	83	32	$\mathbf{b}\mathbf{b}$	43	26
	RR	38	32	Rr	82	32	rr	42	30
	PdgPdg	1.01	32	Pdgpdg	57	29	pdgpd	g 4	28
Oderbrucker×				BB	82	52	bb	82	55
F_1 (Lion 28 \times Oderbrucker)				Ss	82	54	SS	82	53
Lion 28×F				\mathbf{Rr}	42	20	rr	59	20
(Lion 28 ×	******	• •••••		$\mathrm{E_{2}e_{2}}$	52	23	e ₂ e ₂	49	17
Òderbrucker)	*****	******	*****				6969		
$\operatorname{Persicum} imes$	$\mathbf{v}\mathbf{v}$	36	12	Vv	74	12	vv	57	19
Oderbrucker	SS	30	9	Ss	95	16	SS	42	16
	$_{ m BB}$	41	14	Bb	83	15	bb	43	- 15
	RR	41	18	\mathbf{Rr}	84	12	rr	42	15
	$\mathbf{E_2}\mathbf{E_2}$	48	15	$\mathrm{E_{2}e_{2}}$	76	15	e_2e_2	43	13
Oderbrucker×				Vv	68	34	vv	50	: 29
F ₁ (Persicum ×			*****	$\mathbf{B}\mathbf{b}$	57	38	bb	61	28
Oderbrucker)			*****	$\mathbf{S}\mathbf{s}$	72	30	SS	46	37
Persicum \times F.			*****	\mathbf{Rr}	53	3	rr	49	1
(Persicum ×	VV	48	2	$\mathbf{v}_{\mathbf{v}}$	54	2	*****		
Oderbrucker)				E_2e_2	53	2	e_2e_2	49	2
Brachytic×	BrBr	56	9	Brbr	108	8	brbr	30	11
Oderbrucker	SS	53	8	Ss	97	. 10	SS	44	7
Oddi bi dokdi	$\stackrel{\sim}{ m NN}$	54	$1\overset{\circ}{0}$	$\widetilde{\mathbf{N}}\mathbf{n}$	102	9	nn	38	7
Colsess IV ×	BrBr	49	16	Brbr	83	13	brbr	21	17
Brachytic	SS	39	17	Ss	77	13	SS	37	14
maciny mo	NN	48	14	Nn	77	14	nn	28	15
	KK	39	$\overline{15}$	Kk	78	14	kk	36	13
Persicum ×	ВВ	16	. 8	\mathbf{Bb}	64	16	bb	44	24
Iris	RR	54	19	R_{r}	53	13	ı'r	17	25
7119	NN	41	12	Nn	55	19	nn	28	21
	VV	26	7	Vv	54	19	vv	44	$\frac{21}{21}$
	$\mathbf{E_2}\mathbf{E_2}$	42	16	$\mathbf{E_{2}e_{2}}$	52	17	e_2e_2	30	20

stripe reaction, the segregation then fit a 1:2:1 ratio satisfactorily for 2-rowed: heterozygous: non-2-rowed.

a S—rachilla hairs long, s—rachilla hairs short.

E₂—Glume awns long, e₂—glume awns short.

B—black lemma and pericarp, b—white lemma and pericarp.

R—rough awns, r—smooth awns.

Pdg—glumes hairy, pdg—glumes glabrous.

V—non-six-rowed, v—six-rowed.

Br—plants normal, br—plants brachytic.

N—caryopses hulled, n—caroypses naked.

K—hooded, k—awned.



Tests indicated that rough vs. smooth awns and rachilla hair length were associated, with a percentage recombination of 28 ± 3.3 . The independence of lemma color and glume awn length, and row number and glume awn length was questionable. The factors that governed the other characters in Persicum × Oderbrucker were independent. Also there did not appear to be any definite association between the stripe classes and any one of the 5 morphological characters, either according to the Chi-square tests or by observation (Table 3).

For the backcross Oderbrucker $\times F_1$ (Persicum \times Oderbrucker) and its reciprocal, there was some lack of agreement between reciprocals, due perhaps to the small number of lines. The segregation for lemma color indicated monogenic inheritance. For rachilla hair length and row number the segregations of reciprocals did not quite agree, but single factors were suggested. Row number and lemma color were independent of each other. With Oderbrucker as the female, row number and lemma color both appeared to be independent of rachilla hair length, but when the F_1 was the female they did not. According to Chi-square tests each of these 3 characters was independent of the stripe-reaction classes. The averages for the various genotypes substantiate this evidence (Table 3).

For the backcross Persicum \times F_1 (Persicum \times Oderbrucker) and its reciprocal, rough vs. smooth awns, long- vs. short-glume awns, and 2-rowed vs. non-2-rowed were indicated as monogenic, and independent of each other and of the stripe classes. The data in table 3 also suggest this independence.

In table 3 there are some trends of average infection that may represent associations, e.g., in Persicum × Oderbrucker and in F_1 (Persicum × Oderbrucker) × Oderbrucker vv was higher in infection than VV or Vv, but these trends do not seem to be consistent enough to be considered significant. In Oderbrucker × F_1 (Persicum × Oderbrucker) and its reciprocal the heterozygous black lines had higher infection than the homozygous white lines, but this is the opposite of what would be expected if an association were involved.

$Brachytic \times Oderbrucker$

In Brachytic the caryopses are naked when threshed, rachilla hairs are long, and the plants have much shortened internodes. In Oderbrucker the lemma and palea adhere tightly to the caryopses, rachilla hairs are short, and the plants are normal in height.

The brachytic character has been found by Powers (8) and Swenson (15) to be conditioned by a single factor pair. Hulled vs. naked has been reported as a monogenic character by several workers. In the cross Brachytic × Oderbrucker the segregation for brachytic did not fit the expected 1:2:1 ratio satisfactorily because of a deficiency in the brachytic class. Since brachytic plants in general produced fewer kernels than did normal plants there was probably selection against the brachytic character

in picking lines with sufficient seed for the F₃ tests. However, there were enough brachytic lines included in the tests to show any association with stripe resistance if it were definitely present. Rachilla hair length and hull adherence each appeared to be monogenic in their inheritance. Normal vs. brachytic, long- vs. short-haired rachillas, and hulled vs. naked were independent of each other and of stripe-reaction classes. The independence of genotype and stripe classes is indicated by the averages in table 3.

$Colsess~IV \times Brachytic$

In Colsess the hulls are adherent, there are hoods instead of awns, rachilla hairs are short, and the plants are normal in height. Hooded vs. awned has been reported as being conditioned by 1 factor pair.

TABLE 4.—Distribution of F_3 lines of Colsess $IV \times Brachytic$ for reaction to stripe and covered smut.^a Lines placed according to average of 4 tests for each disease

Covered smut						St	ripe			
Lines in percentage	Lines in percentage infection classes ^b						Total			
infection classes	0	5	15	25	35	45	55	65	75	
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
0	9	15	12	2	1	0	0 .	1	1	41
5	18	17	12	2	0	1	1	4	1	56
15	4	5	11	4	0	. 2	2	0 -	2	30
25	1	4	3	1	-0	1	0	0	0	10
35	1	1	1	0	1 .	1	0	0.	. 0	5
45	2	. 1	1	0	0	0	0 - 1	. 0.	0	4
55	0	1	1	0	0	0	0	0	0	2
65	0	0	0	0	0	0	0	0	0	0
75	1	1	- 1	0	0	0	0	0	0	. 3
85	1	1	0	0	0	0	0	0	0	2
Total	37	46	42	9	2	5	3	5	4	153

a Covered smut reactions supplied by R. G. Shands.

For the cross Colsess IV × Brachytic segregations for hooded vs. awned and long- vs. short-haired rachillas each fit the single factor assumptions, while hulled vs. naked and normal vs. brachytic did not. The factors governing the inheritance of these 4 characters were independent of each other and of the stripe classes. The independence of these characters and stripe reaction is also indicated by the averages in table 3.

The lines of this cross had been previously tested for reaction to covered smut (*Ustilago hordei* (Pers.) K. & S.) by R. G. Shands. The distribution for reaction to stripe and smut appears in table 4. For the diseases individually the distributions were very similar. There were no lines highly susceptible to both diseases. There was no correlation between the reactions to the 2 diseases.

b Each line was tested 4 times for each disease, and placed on the basis of the average of the 4 tests.

$Persicum \times Iris$

Iris has a white lemma and pericarp, rough awns, naked caryopses, long glume awns, and is 6-rowed. Persicum carried the alleles for the factors governing the inheritance of each of these characters.

Hulled vs. naked and long vs. short awns each gave satisfactory agreement with 1:2:1 segregation expected on the basis of single factor differences. Segregations for black vs. white, rough vs. smooth awns, and 2-rowed vs. non-2-rowed did not fit those expected for monogenic inheritance. These 5 characters were independent of each other, except hulled and 2-rowed. They were all independent of stripe-reaction classes. The average stripe infections for the various genotypes are in table 3. These seem to suggest some association between stripe resistance and black lemma color, and between stripe resistance and 2-rowed, even though the Chisquare tests did not detect them.

DISCUSSION

The data on the inheritance of stripe reaction have shown that there were genetic differences between varieties which appeared to be similar in their reaction to the disease. In varietal tests Oderbrucker has appeared to be as susceptible as Colsess and Iris, but in crosses with resistant varieties the susceptibility of Oderbrucker was recovered with less frequency than that of Colsess or Iris. The distributions of F3 lines in order of increasing average infection were rather distinctly different for Persi- $\operatorname{cum} \times \operatorname{Oderbrucker}$ and $\operatorname{Persicum} \times \operatorname{Iris}$ as shown in figure 3, in that there was a definite break in direction in the Persicum × Iris distribution at the 25 per cent level which was not present in the $\operatorname{Persicum} \times \operatorname{Oderbrucker}$ distribution. Since the resistant parent was the same in both crosses there must have been a genetic difference between Oderbrucker and Iris. Persicum and Brachytic have been shown to have some factors in common for resistance. On the basis of the similarity of the distributions of Colsess \times Brachytic and Persicum \times Iris it might be concluded that Colsess and Iris have some factors for susceptibility in common.

Attempts to find associations between stripe reaction and certain morphological characters have not been successful. Marker genes in at least two linkage groups were involved in each cross, and among the various crosses marker genes in six of the seven linkage groups of barley, number VI excepted, have been tested for their relationship to stripe reaction (Table 5). However, since the resistances were genetically different and apparently the susceptibilities also, independence in one cross does not justify the conclusion of independence in another cross where that particular marker gene could not be studied. A cross involving marker genes in all linkage groups, as well as differences in stripe reaction, would be helpful in this respect.

The factors conditioning stripe reaction may have been located in other linkage groups than those investigated in any particular cross. Since a

number of factors seemed to be involved it might be more logical to conclude that they were located on several chromosomes, and that they all had approximately equal effects in conditioning resistance. It is also possible that the genes affecting stripe reaction were so far removed from the marker genes on the chromosome that the linkage did not become evident with the methods employed.

TABLE 5.—Summary of genes and linkage groups studied in various crosses with reference to their stripe reaction

Linkage group	Lion × Oder- brucker	Persicum × Oder- brucker	Brachytic × Oder- brucker	Colsess IV × Břachytic	Persicum ×Iris
I II IV	Bb	Vv Bb	Nn.	Nn Kk	Vv Bb Nn
v	$\operatorname{Rr}\operatorname{Ss}$	$\operatorname{Rr}\operatorname{Ss}$			Rr
VII			Br br	Br br	

A key to the symbols will be found in the footnote of table 3.

SUMMARY

Two types of resistance and two types of susceptibility to the stripe disease were apparent in the varieties considered. The differences in type were evident from the varietal reactions and from the effects of the differences on the progenies of crosses. Persicum and Brachytic remained highly resistant to the culture of the fungus used. The modes of inheritance indicated that these two varieties had some factors for resistance in common. In crosses with Oderbrucker, resistance appeared to be dominant and three factors were probably involved. The resistance of Lion was not complete, and was apparently inherited in a manner different from that of Persicum. Dominance was not definite and a number of factors seemed to be involved. The susceptibility of Oderbrucker was probably different from that of Colsess and Iris, as in the crosses Persicum × Iris and Colsess × Brachytic a difference of one major and one modifying factor pair was involved in each case.

Marker genes in six of the seven linkage groups present in barley were tested for their relationship to stripe reaction, but no associations were found.

Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

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STUDIES ON CRANBERRY FALSE BLOSSOM

L. O. KUNKEL

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INTRODUCTION

During the Christmas Meetings of the American Association for the Advancement of Science at Philadelphia, in 1940, the writer exhibited periwinkle (Vinca rosea L.) plants that had been cured of aster yellows (Chlorogenus callistephi var. vulgaris Holmes) by heat treatments (14). Dr. Neil E. Stevens visited the exhibit and suggested that similar treatments should be tried on cranberry plants (Vaccinium macrocarpon Ait.) affected by false blossom (Chlorogenus vaccinii Holmes). Later he kindly offered to supply diseased cranberries for this purpose. His offer was accepted and on June 11, 1941, about sixty false-blossom plants were delivered to The Rockefeller Institute for Medical Research at Princeton, New Jersey, by Dr. Stevens' son, Mr. Russell B. Stevens. My indebtedness to both is gratefully acknowledged.

Preliminary experiments directed toward a determination of ability of false-blossom plants to endure heat were undertaken immediately. However, cranberries were not well suited for heat-treatment tests. Most of the plants that survived the treatments did not resume growth for several months. A few that started growing soon after treatment produced new foliage slowly and did not blossom. Since foliage symptoms of this disease are not very distinctive it was impossible to say whether any of the treated plants had been cured. It was realized that considerable time and a rather large number of plants would be required to make an adequate study of any effects that heat might have on false blossom in cranberry. It was believed that a preliminary study of thermal relationships of the virus in a host that might prove more suitable than cranberry would be advisable. Therefore an attempt was made to transmit the virus to herbaceous plants.

At the time the work was under contemplation the only known vector of false blossom was the cranberry leafhopper Euscelis striatulus (Fallen) (5). As this insect was not readily available and as nothing was known regarding its ability to feed on periwinkle or other herbaceous plants, it was decided to try transmission by dodder, Cuscuta campestris Yuncker (1, 10, 11), which had been shown to transmit several other viruses. It was soon found that dodder would transmit false blossom to periwinkle. This permitted investigation of effects of heat on the disease in a plant that was more suitable for experimental purposes than cranberry. It also made available a means of studying other phases of the false blossom problem. Some of the work already has been reported briefly (15, 16, 17, 19). It will be presented in detail in this paper.

MATERIALS AND METHODS

The source of the virus used in all experiments was the diseased cranberry plants received from Dr. Stevens. All healthy cranberry plants needed in the work were grown from seeds obtained from fruits purchased in grocery stores in Princeton, New Jersey. Periwinkle plants were grown from cuttings taken from plants belonging to a clone. The dodder used was from seeds obtained from a virus-free plant of *Cuscuta campestris* growing on a healthy sugar-beet plant. Other species were obtained from seed purchased from commercial seed companies.

All plants were grown in porous clay pots set on benches in greenhouses. The houses were fumigated at weekly intervals to control insects. Cranberries were grown in a mixture of equal parts of sand and peat, other plants in rich garden earth. Heat treatments were given in a room that was described previously (13).

Virus-free dodder was kept growing on healthy tomato or sugar beet plants at all times. When it was needed for experiments, branches from four to six inches long were broken off and placed on plants to be parasitized. Usually only one dodder branch was used in making a transfer. The tip of a dodder branch was placed in the tip of the new host. The dodder usually entwined a stem or leaf petiole within 24 hours, but occasionally it failed to take hold and fell out of the new plant. When this happened it always was put back on the plant from which it had fallen.

TRANSMISSION OF FALSE BLOSSOM BY DODDER

Transmission from cranberry to tomato and periwinkle.—In experiments during the autumn of 1940 and during 1941 false-blossom virus was transmitted from cranberries to several hundred tomato and periwinkle plants. There was at the time some uncertainty as to whether the virus being transmitted was that of false blossom; the possibility that another virus might be present in diseased plants had to be considered. Some evidence against that view was obtained from experiments showing that the virus taken to tomato and periwinkle could invariably be gotten from false-blossom plants. All question as to identity was resolved when the virus was taken from tomato and periwinkle to healthy cranberries and shown to produce typical false-blossom symptoms. A few transmission experiments will be described in detail.

Virus-free dodder was transferred from a healthy tomato plant to 8 false-blossom cranberry plants and to 8 healthy cranberry plants. It was allowed to parasitize the cranberry plants for six weeks. A small branch was then transferred from each cranberry plant to a healthy tomato plant. The dodder was allowed to parasitize the tomato plants for six weeks when it was removed and destroyed. Five of the 8 tomato plants exposed to dodder from false-blossom plants came down with false blossom. The other 3 plants and the 8 plants exposed to dodder from healthy cranberry plants remained healthy in appearance for the 9 months that they were

under observation. The experiment showed that dodder transmitted virus from false-blossom cranberry plants to tomatoes.

Several branches of dodder from false-blossom cranberry plants were placed on each of 6 healthy young plants of *Vinca rosea*. Similar branches from healthy plants were placed on 6 other plants of *Vinca rosea* that were to serve as controls. Two weeks later all of the dodder was removed and destroyed. Sixteen days thereafter clearing of veins appeared in leaves near the tips of two branches on one plant of *Vinca rosea* that had been parasitized by dodder from the false-blossom plants. Soon other false-blossom symptoms appeared in these and other branches of the plant. During the next 2 weeks 4 of the 5 other plants of *Vinca rosea* that had been exposed to false-blossom dodder came down with false blossom. The other plant did not show symptoms of disease until 87 days after it had been exposed to viruliferous dodder. The 6 control plants remained healthy. The experiment showed that virus could be transmitted from false-blossom cranberry plants to *Vinca rosea* by dodder.

In another experiment virus-free dodder was transferred to 30 falseblossom cranberry plants and to 30 healthy cranberry plants. It was allowed to parasitize the plants for three months. Then small branches were transferred from the 60 cranberry plants to 60 sets of healthy plants consisting of one tomato and one periwinkle each. In most instances the branches quickly established unions with the new hosts and grew rapidly. In all cases in which this did not occur more branches from the same sources subsequently were placed on the plants. After the dodder had parasitized the tomato and periwinkle plants for about one month it was removed and destroyed. Of the 30 tomato and 30 periwinkle plants exposed to dodder from healthy cranberry plants all remained healthy up to the time the experiment was ended. Of the 30 tomato plants exposed to dodder from false-blossom cranberry plants all but 6 were diseased, and of the 30 periwinkles exposed to dodder from false-blossom cranberry plants all but 9 were diseased within 32 days after the dodder was removed. The 6 healthyappearing tomatoes and the 9 healthy-appearing periwinkles were then cut back, as pollarding had been found to hasten the appearance of symptoms. Within 7 weeks after this was done all of the plants were diseased. experiment showed that virus could be transmitted readily from falseblossom cranberry plants to tomatoes and periwinkles.

Transmission from tomato to tomato.—Virus-free dodder was placed on a healthy and a false-blossom tomato plant. After the dodder had parasitized the plants for 3 weeks small branches from the diseased tomato were transferred to each of 13 healthy young tomato plants. Branches of dodder from the healthy tomato were transferred to each of 3 other healthy young tomato plants. The dodder was left on the plants for about 2 months, when it was removed and destroyed. In due course all of the 13 plants exposed to dodder from the diseased tomato became diseased. The control plants exposed to dodder from the healthy tomato remained healthy.

The experiment showed that false-blossom virus could be transferred readily from tomato to tomato by dodder.

Transmission from tomato and periwinkle to cranberry.—Virus-free dodder was placed on a healthy and on a false-blossom tomato plant. When it had parasitized the plants for about 6 weeks small branches from the diseased tomato were placed on each of 8 healthy cranberry plants. Branches from the healthy tomato were placed on each of 8 other healthy cranberry plants. After the dodder had parasitized the cranberry plants for 33 days it was removed and destroyed. Forty days later the cranberry plants were examined and no symptoms of disease were found. It was realized that symptoms might be slow to appear in the cranberry. Hence it was decided to determine whether or not virus could be obtained from any of the plants.

Virus-free dodder was placed on each of the 16 cranberry plants. After it had parasitized the plants for 6 days small branches were transferred to 16 healthy young tomato plants. After the dodder had grown on the tomato plants for 14 days it was removed and destroyed. Twenty-six days later early symptoms of false blossom appeared in one of the tomato plants that had been exposed to dodder from a cranberry that had been parasitized by false-blossom dodder. Eventually 6 of the 8 tomato plants thus exposed became diseased. The other 2 tomato plants and the 8 tomato plants exposed to dodder from the control cranberry plants remained healthy. The test showed that virus could be obtained from 6 of the 8 cranberry plants that had been parasitized by dodder from diseased tomato plants. About 6 months after this test was made the 6 cranberry plants from which virus was obtained had well-marked symptoms of false blossom. During the following year 4 of the 6 diseased cranberry plants produced flowers with typical false-blossom symptoms; the other 2 diseased cranberry plants did not flower. The experiment proved that false-blossom virus can be transmitted from tomato to cranberry by dodder.

Virus-free dodder was placed on 5 false-blossom and on 5 healthy periwinkle plants. When the dodder had parasitized the periwinkle plants for 3 months it was transferred to 10 healthy cranberry plants. After growing on these plants for 3 months it was removed and destroyed. During the following year all of the 5 cranberry plants on which dodder from diseased periwinkles had been placed had well-marked foliage symptoms of false blossom; one of the plants produced flowers and fruits with typical symptoms of false blossom. The 5 cranberry plants on which dodder from healthy periwinkles was placed remained healthy. The experiment showed that false-blossom virus can be transmitted from periwinkles to cranberries by dodder.

Transmission to other species.—As the work on false blossom was undertaken for the purpose of investigating any beneficial effects that heat might have on diseased plants, no special effort was made to transmit the virus widely. However, attempts were made to take it to such species as were

available at the time it was being transmitted to *Vinca rosea*. The plants that took the disease when exposed to virus-bearing dodder are listed:

at took the disease when exposed to virus-bea	ring dodder are listed:
Dianthus sp., var. Crown of Perfection	in the Caryophyllaceae
Eschscholzia californica Cham.	Papaveraceae
Pastinaca sativa L., Parsnip	Umbelliferae
Apium graveolens L., Celery, var. Silver	
self blanching	do
Petroselinum hortense Hoffm., Parsley	do
Daucus carota L., Cultivated carrot	do
Vaccinium macrocarpon Ait., Cranberry	Ericaceae
Vinca rosea L., Periwinkle	Apocynaceae
Phlox Drummondii Hook.	Polemoniaceae
Petunia hybrida Hort.	Solanaceae
Nierembergia frutescens Dur.	do
Schizanthus sp.	do
$Salpiglossis\ sp.$	do
Lycopersicon esculentum Mill., Tomato	do
Nicotiana tabacum L., Turkish tobacco	do
Nicotiana glutinosa L.	do
Nicotiana langsdorffii Schrank	đo
Nicotiana rustica L.	do
Solanum tuberosum L., Potato	do
Veronica peregrina L.	Scrophulariaceae
Scabiosa atropurpurea L.	Dipsaceae
Dimorphotheca aurantiacum DC, African	
daisy	Compositae
Brachycome iberidifolia Benth., Swan	
River daisy	đo
Centaurea imperialis Hort.	do
Gaillardia aristata Pursh	do
Tagetes erecta L., African marigold	do .
Calendula officinalis L.	do
Tragopogon porrifolius L., Salsify, var.	
Sandwich Island	do

Some plants that did not take the disease when exposed to virus-bearing dodder are the following:

Begonia sp.	in the Begoniaceae
Prunus persica Stokes, Peach	Rosaceae
Medicago sativa L., Alfalfa	Leguminosae
Callistephus chinensis Nees, China aster	Compositae

The disease was taken to 28 species belonging in 10 different families of plants. The families represented are not closely related. The lowest plant in our scheme of classification is in the pink family and the highest in the Compositae. The species that failed to become diseased were exposed

to virus-bearing dodder for at least 3 months and several alfalfa plants were exposed for more than a year. In each case virus-bearing dodder was replaced by virus-free dodder that was allowed to parasitize the plants for 3 months. At the end of this period the dodder was shown to have remained virus-free. It is presumed that alfalfa and the other 3 plants that did not take the disease are immune.

TRANSMISSION OF FALSE BLOSSOM BY GRAFTING

Cranberry vines are too thin to be grafted readily; it probably is because of this that experimental transmission of false blossom by grafting has not been reported previously. Bergman and Truran (2) observed an apparent case of cranberry false-blossom virus transmission through a natural graft. The writer attempted to transmit the virus in cranberry by grafting diseased scions onto healthy plants, but did not succeed, undoubtedly because the scions did not live long enough to form unions with the stocks. During the past three years false blossom has been passed in series in Vinca rosea and in a number of different solanaceous plants by grafting, the whip graft being employed. The plants from which and to which the virus was taken by grafting are the following: from Vinca rosea to Vinca rosea; from tomato to tomato. Turkish tobacco, and Nicotiana rustica; from Turkish tobacco, potato, and Nicotiana glutinosa to tomato; from Nicotiana rustica to Nicotiana rustica and Nicotiana glutinosa; from potato to potato; and, from Nicotiana glutinosa to Nicotiana glutinosa, The minimum period recorded for first appearance of false-blossom symptoms after grafting a plant was 23 days in an experiment in which diseased tomato scions were grafted to healthy tomato plants. The usual incubation period in graft transmissions was about 40 days.

MULTIPLICATION OF VIRUS IN DODDER

Infective dodder retained the virus and remained infective for several months even on plants that were immune from the disease. Viruliferous dodder that was allowed to grow on aster, Begonia, and alfalfa for 3 months or longer retained its ability to infect tomato and carrot, although the aster, Begonia, and alfalfa plants remained healthy in appearance, and virus-free dodder allowed to parasitize them did not become infected. This showed that the plants were not symptomless carriers and suggested that the virus might multiply in the dodder. By using small branches in making successive transfers of infective dodder on alfalfa plants Costa (3) showed that infectivity was retained beyond a point where it should have been lost through dilution by growth if there had been no multiplication. From this evidence he concluded that the virus multiplies in dodder. Similar experiments carried out by the writer confirmed this view. In the most thoroughgoing test that was made dodder was passed successively to 5 healthy tomato and 5 healthy alfalfa plants during a period of about 2 years. In each instance a branch of dodder approximately four inches long was used in making a transfer. The test was started on October 27, 1942, when a branch of dodder produced on a false-blossom cranberry plant was placed on tomato plant number 1. After the parasite had made good growth on tomato number 1 a branch was used to transfer it to tomato number 2 on November 17, 1942; similarly, a branch was transferred from tomato number 2 to tomato number 3 on December 8, from tomato number 3 to tomato number 4 on December 29, and from tomato number 4 to tomato number 5 on January 16, 1943. Successive transfers were at intervals of about 3 weeks. It was believed that the dodder would not be able to obtain virus from the tomato plants within so short a period after their exposure to infection and that if the parasite remained infected it would mean either that the virus multiplied in it or had not been sufficiently diluted by growth to prevent transmission. During the time the dodder was being transferred from tomato to tomato evidence that alfalfa was immune to infection by false blossom became available. Hence alfalfa was substituted for tomato when the 6th successive transfer was made on February 6, 1943. falfa plants also were used when the 7th, 8th, 9th, and 10th successive transfers were made on April 6 and May 10, 1943, and on April 15 and July 13, 1944, respectively. The periods between transfers to alfalfa plants were about 2, 1, 11, and 3 months, respectively. Each time a transfer was made to a new tomato or alfalfa plant transfers also were made to healthy carrot plants in order to determine whether the dodder had remained infective. It was shown to be infective at the time of each transfer. In the final test dodder from alfalfa plant number 5, on which it had been growing for almost 3 months, was transferred to 10 carrot plants on November 10, 1944. On January 26, 1945, all of the 10 carrots had well-marked symptoms of false blossom. The virus was retained over the entire period and there was no indication that any decrease in the infectivity of the dodder occurred.

It was estimated that on tomato the dodder increased in size, at least, tenfold between each transfer. Thus after parasitizing the 5th tomato plant the virus should have been diluted by growth to 10⁻⁵, at least, in case there had been no multiplication in the dodder. The dodder increased more than tenfold on each of the 5 alfalfa plants. Its tendency to overgrow the plants was held in check by frequent and rather severe prunings. If the dodder increased only tenfold per month during the 20 months it was grown on the alfalfa plants the virus should have been diluted to 10⁻²⁰ of the concentration obtaining in the branch taken from the 5th tomato plant. Since the virus in that branch already had been diluted to 10⁻⁵ of the concentration in the branch from the diseased cranberry, the virus in the branches used in the final test should have contained not more than 10⁻²⁵ of the concentration in the original branch if there had been no multiplication. It seemed unlikely that virus at such a high dilution would give infection even through a dodder union. Hence it was concluded that falseblossom virus multiplied in the dodder. This conclusion was reached with

some reluctance because there was no evidence that on healthy plants infective dodder grew less vigorously than virus-free dodder. Moreover, viruliferous dodder bore normal flowers and viable seeds, and showed no symptoms of disease at any time.

SYMPTOMS

The symptoms of false blossom in cranberry are well known. Some of those observed in tomato, periwinkle, Calendula, and Nicotiana glutinosa have been described and illustrated elsewhere (16, 17). Hence it seems unnecessary to describe the symptoms produced in the other species to which it was taken. In many plants, including tomato, the virus produced characteristic symptoms in the reproductive organs. It caused virescence, distortion, and gigantism in some or all of the flowering parts. The virus of aster yellows in the China aster and the viruses of several other diseases in the yellows group sometimes cause enlargement of flowers of host plants, but no other virus with which the writer is acquainted produces flower symptoms comparable with those of false blossom. Enlargement and fusion of sepals in flowers borne by false-blossom tomato plants produced the structures characteristic of the disease known as big bud. This, of course, does not prove that false blossom and big bud are closely related. Flower symptoms were not observed in celery, parsley, parsnip, and salsify because the plants used were not old enough to bear flowers at the time they became diseased. Flower symptoms were observed in all other plants to which the virus was transmitted. In some species it caused much less stimulation of flowering and fruiting organs than in others; for example, infected flowers in Nicotiana rustica were not greatly enlarged (Fig. 1). The flowering branch shown in the upper row at the left is healthy; all others are diseased and show different degrees of distortion. All species bore flowers that were smaller than normal after they had been diseased for some time. It should be clearly understood that big-bud symptoms in any host of false blossom are onset symptoms, or at least symptoms that appear only during the time the infection is becoming established. In late stages of disease the plants usually bore no flowers whatever. The virus caused all plants to become chlorotic, to assume a more upright habit of growth than is normal, and to produce large numbers of secondary shoots. In general it may be said that false blossom is a yellows type disease that causes marked and specific effects on flowering and fruiting organs.

STRAINS OF FALSE BLOSSOM VIRUS

False-blossom cranberry plants received from Dr. Stevens showed different degrees of severity; some were stunted much more than others. Leaves on badly stunted plants usually were smaller than leaves on plants that had made better growth. At first it was presumed that the severely stunted plants had been diseased for a longer time than larger plants. However, when virus was transmitted from eranberry plants showing dif-

ferent degrees of stunting to periwinkle and tomato plants it was observed that in the latter also the disease showed different degrees of severity. Virus transmittd from badly stunted cranberry plants produced severe stunting in periwinkle and tomato, whereas virus transmitted from less seriously affected cranberry plants produced less stunting in periwinkle and tomato. When tomato plants affected by the viruses from several different cranberry plants were arranged in a row according to severity of stunting they presented a graded series of symptoms. This observation suggested that strains of one virus, rather than several different viruses, were causing the different types of disease. Two of the strains were trans-

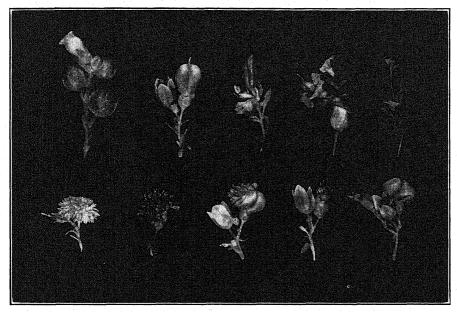


Fig. 1. Flowers and fruiting capsules of *Nicotiana rustica* affected by false blossom, Branch in upper row at left is healthy; all others are diseased. The picture shows malformations caused by false blossom in this host. (Photograph by J. A. Carlile.)

mitted in series in periwinkle by grafting. In four successive transfers each strain remained unchanged as judged by the symptoms produced (Fig. 2, A). However, on a plant that was kept under observation for about six months and was affected by the milder of the two strains a branch developed that showed severe symptoms. No branches on the plants affected by the severe strain developed mild symptoms. This aspect of the cranberry false-blossom disease problem deserves further study.

FALSE BLOSSOM NOT TRANSMITTED BY MEANS OF JUICE

It is a well-known fact that false blossom cannot be transmitted to cranberry plants by means of juice inoculations. Nevertheless the possibility existed that it might be transmissible to other species by this means. When it was found that the disease would go to solanaceous plants, at-

tempts were made to transmit it to two host species by the rubbing method of inoculation. Since all attempts to pass the virus in this way were unsuccessful only one experiment will be described.

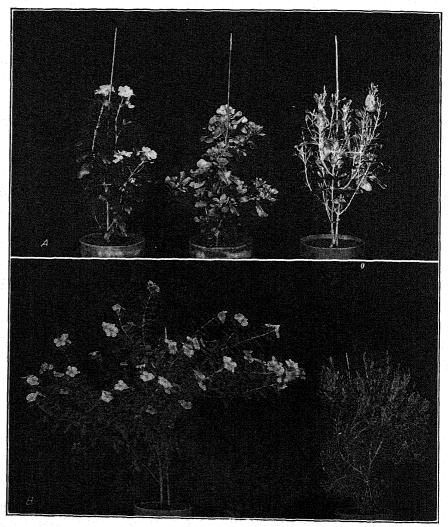


Fig. 2. A. Three periwinkle plants of the same age. Plant at the left is healthy, plant in the middle is affected by a mild strain, and plant at the right by a severe strain of false-blossom virus. B. Two periwinkle plants of the same age. Both were badly affected by false blossom when the plant at the left was given a 21-day treatment at 42° C. The picture was made about 5 months after treatment; it shows that the treated plant was cured and that the untreated plant remained badly diseased. (Photographs by J. A. Carlile.)

Juice was pressed out of leaves and stems of a false-blossom tomato plant and immediately rubbed over the upper surfaces of all leaves of 6 healthy tomato plants and 2 healthy plants of *Nicotiana glutinosa*. Juice from a healthy tomato plant was rubbed over the upper surfaces of the

leaves of 2 healthy tomato plants and 1 healthy plant of Nicotiana glutinosa that served as controls. All of the plants remained healthy in appearance during 3 months that they were kept under observation. It was concluded that the virus could not be transmitted to these species readily, if at all, by means of juice.

FALSE BLOSSOM NOT TRANSMITTED BY MACROSTELES DIVISUS (UHL.)

Although false blossom is a yellows type disease it seemed unlikely that it would be transmitted by the aster yellows vector, *Macrosteles divisus*. Nevertheless this possibility was tested in 3 experiments. In one of these, 65 virus-free nymphs were allowed to feed on a false-blossom carrot plant for 12 days. A similar virus-free colony of the same size was kept on a healthy carrot plant for the same period. The 2 colonies were held on 2 different aster plants for 5 days. Then each colony was passed successively to 11 different healthy carrot plants. They were confined on the plants for intervals varying from 2 to 12 days during a period totaling 40 days. Only 3 insects from the colony that had fed on the false-blossom carrot plant, and only 5 insects from the control colony were living when the test was ended. All plants were observed for 3 months following exposure to insects. They all remained healthy. It was concluded that *Macrosteles divisus* is unable to transmit cranberry false blossom in carrots.

FALSE BLOSSOM NOT TRANSMITTED THROUGH DODDER SEEDS

Seeds were collected from a large dodder plant that had grown and produced seeds on a false-blossom tomato. Many of the seeds were shrivelled and more or less deformed, but some were plump. Whether the poor quality of the seeds produced was due to infection of the dodder by false blossom virus or to insufficient nourishment furnished by the diseased host is not known. One hundred of the best-looking seeds were selected and planted in moist peat; only 6 of them germinated. When the seedlings were about one and one-half inches high each was transferred to a different healthy young tomato plant. All of the dodders grew and parasitized the plants on which they had been placed. After they had fed on the plants for 3 months they were removed and destroyed. The tomato plants were cut back and kept for 4 months after the dodders were removed. All remained healthy. Although the number of seedlings grown and tested was small, it was concluded that the virus cannot be transmitted readily, if at all, through dodder seeds.

An effort was made to determine whether it might be transmitted through tomato seeds. If Bonny Best tomato plants are large enough to be setting fruit at the time they become infected with false-blossom virus they will produce undersized fruits that ripen more or less normally. Such fruits usually bear no seeds but some contain fair numbers of small seeds and a few seeds that are almost but not quite as large as those borne by healthy fruits. Out of fruits that were harvested from 30 diseased tomato

plants 25 fairly normal looking seeds were obtained. They were planted in soil in two 8-inch pots. None of them germinated; hence it was not possible to determine whether or not they carried virus.

CURE OF FALSE BLOSSOM IN PERIWINKLES

A preliminary heat treatment experiment in which false-blossom periwinkles were held at 34° C. for periods up to 2 weeks gave no cures; hence, higher temperatures were tried. Eighteen diseased plants were divided into 9 sets of 2 plants each; 1 set was not treated and served as a control. The other 8 sets were treated at 36° C. for 7, 8, 9, 10, 11, 12, 13, and 14 days, respectively. A month after treatment it was observed that the tops of the plants exposed to heat for 7 and 8 days were somewhat greener than the tops of control plants, but that the new growth had symptoms of disease. The tops of the plants exposed for 9, 10, 11, and 12 days had produced new growth bearing normal flowers, and foliage that appeared healthy. However, sprouts that had developed on the main stems and large branches at some distance below their tips had false-blossom symptoms. The tops of the plants exposed for 13 and 14 days appeared healthy throughout, but sprouts arising at or slightly above the ground level showed symptoms in all cases. The tops of these plants apparently had been cured but virus had remained in the underground stem tissues, and, no doubt, in the roots.

In the next experiment 12 diseased plants were divided into 6 sets of 2 plants each. One set was not treated and served as a control. The other 5 sets were treated at 40° C. for 7, 8, 10, 12, and 14 days, respectively. The condition of the plants was observed and recorded 2 months after treatment. Those treated for 7 and 8 days had false-blossom symptoms in many of their branches but the tips of some branches bore normal flowers and healthy-appearing leaves. The tips of all branches on the plants exposed for 10 days were healthy in appearance, but the main stems and large branches bore diseased sprouts a few inches below the tips. The new growth in the tops of the plants treated for 12 days was healthy in appearance, but each plant bore diseased sprouts that arose near the ground level. The tops of these plants apparently had been cured but virus persisted in the underground parts. All new growth produced by the periwinkles treated for 14 days was healthy in appearance and remained so for the 8 months that the plants were under observation. It was concluded that they had been cured. The experiment showed that treatment of 40° C. for 12 days cured the tops but not the underground parts, while treatment at 40° C. for 14 days cured both tops and underground parts.

In another experiment diseased plants were treated at 42° C. for 14 days or longer. Ten diseased plants were divided into 5 sets of 2 plants each. One of the sets was not treated and served as a control; the other 4 sets were treated for 14, 16, 18, and 21 days, respectively. All of the plants were observed for 8 months. At no time did symptoms of disease

appear in new growth from the treated plants. The control plants were diseased throughout (Fig. 2, B). It was concluded that all of the treated plants in this experiment were cured.

CURE OF FALSE BLOSSOM IN CRANBERRIES

As has already been stated, preliminary heat treatment experiments with false-blossom cranberries were carried out soon after diseased plants were received. In one experiment 8 plants were exposed to 36° C. for periods varying by one day intervals from 7 to 15 days. None of the plants was seriously injured but none was cured. In another experiment 4 plants were treated at 38° C., and 4 at 40° C., for periods varying by 7-day intervals from 1 to 4 weeks. All of the plants treated for 2, 3, and 4 weeks were seriously injured and later died. The plants treated for one week lived and after some time produced new growth in which false-blossom symptoms eventually appeared.

When the results with periwinkles became available further heat treatments with cranberries were undertaken. The early cranberry experiments had indicated that heat treatments at fairly high temperatures for periods up to about 10 days caused less injury than treatments at somewhat lower temperatures for longer periods. The periwinkle experiments had shown that treatment at 40° C. had to be continued for about 2 weeks to inactivate all of the virus in underground parts. Therefore, treatments at 42° and 43° C. were tried.

While the work was in progress Stevens (26) published a brief note describing unsuccessful attempts to cure false blossom. He subjected dormant false-blossom plants to temperatures varying from 118° F. to 129° F. His periods of treatment varied from 5 minutes to 2 hours for the higher temperatures and up to 12 hours for the lower temperatures. He states that temperatures above 122° F. for periods longer than 30 minutes killed or hopelessly stunted the plants, whereas temperatures below 122° F. even after periods up to 3 hours or more failed to injure the virus.

In the first experiment diseased cranberries were held at 42° C. Three plants were treated for 5 days, 3 for 8 days, 3 for 9 days, and 3 for 10 days. Two of the plants treated for 9 days and 2 treated for 10 days died; all of the others lived and were held under observation for 2 years. The 3 plants treated for 5 days were not cured, as was shown by cranberry false-blossom symptoms that appeared in the new growth, and by transmission of virus from each to tomato plants. The other 5 plants showed no symptoms of false blossom (Fig. 3, A) in growth that developed after treatment, and dodder that was allowed to parasitize them did not transmit virus to tomato plants.

In another experiment 12 diseased cranberries were treated at 43° C. for one week. The plants were injured somewhat and 3 died about a month following treatment. The other 9 plants were held under observation for 2 years. Two of them showed symptoms of false blossom in new growth

about 10 months after treatment, and dodder allowed to parasitize them transmitted false-blossom virus to tomato. The other 7 plants did not show symptoms of false blossom, and dodder allowed to parasitize them did not transmit virus to tomato. Cuttings made from each of them produced healthy-appearing plants.

In another experiment 4 diseased plants were treated at 43° C. for 9 days, 4 for 10 days, and 4 for 11 days. All of the plants were rather seriously injured and most of them died. However, 2 treated for 9 days, 1 treated for 10 days, and 1 treated for 11 days survived. These plants

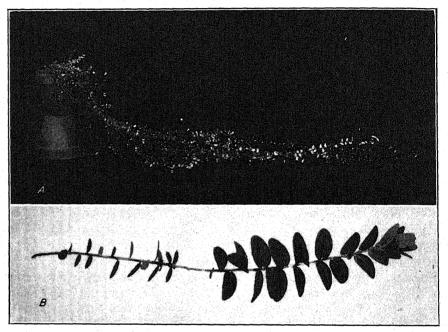


Fig. 3. A. Cranberry plant cured of false blossom by a 10-day treatment at 42° C. The picture was taken about 14 months after treatment. B. Cranberry branch showing recovery from false blossom following a 9-day treatment at 43° C. The small leaves on the older portion of the branch were produced before treatment, the large leaves on the tip portion after treatment. (Photographs by J. A. Carlile.)

showed no symptoms of false blossom in new growth in the 2 years that they were held under observation, and dodder allowed to parasitize them did not transmit virus to tomatoes. The portions of the vines produced by these plants before treatment bore small narrow leaves such as are produced by plants with false blossom, while the tip portions bore large broad leaves such as are produced by healthy plants (Fig. 3, B).

In the three experiments described a total of 16 false-blossom cranberry plants that were treated for varying periods at 42° and 43° C. seem to have been cured. It is known that certain viruses, as for example peach yellows, may be present in the lower parts of plants for a long time without moving into the tops (12). Even tobacco-mosaic virus is slow in

moving from roots into tops of tobacco plants (21). The possibility that false-blossom virus may have remained in the underground portions in some or all of the cranberry plants that apparently were cured must be admitted. The aboveground portions of the plants undoubtedly were cured.

DISCUSSION

Although cranberry false blossom has been known to science for about thirty-five years and is of considerable economic importance (25) the nature of the disease was not fully established until 1929, when Dobroscky (5) found that it was caused by a virus transmitted by the blunt-nosed leafhopper Euscelis striatulus (Fallen). Previous to that time false blossom had been thought by some to result from a physiological disturbance, possibly a disturbance in nitrogen nutrition (24). Dobroscky (6), using the leafhopper vector, attempted to transmit false blossom to the blueberry, Vaccinium corymbosum L., but did not succeed. Up to the time dodder transmission was reported in 1942 the disease had not been seen on any species except the American cranberry, Vaccinium macrocarpon Ait., and the European cranberry, Vaccinium oxycoccus L. (15). It has now been transmitted by dodder to species belonging in ten different families of plants. Thus another plant virus that, for a long time, was thought to be highly specific has been found capable of infecting many species that are not closely related to the natural host. Whether the disease in nature occurs on any of these plants is not known with certainty, but it seems probable that a tomato disease prevalent in Australia and generally known under the name of big bud is identical with or closely related to false blossom. Samuel, Bald, and Eardley (23) state that "The disease that presents the closest parallel to tomato big bud as regards the host plant is undoubtedly cranberry false blossom, transmitted by the jassid Euscelis striatulus." At the time this statement was made false blossom had not been taken to tomato; hence it was not known that it would produce typical big-bud symptoms. The Australian disease is spread by the leafhopper Thamnotettix argentata (Evans) (8). Similar big-bud diseases of tomato also occur in the western part of the United States (4) and in Russia (22), where they are known under the names big bud and stolbur, respectively. The vector or vectors of these diseases are unknown. Another similar disease affects eggplant in southern India, where it has been reported under the name of little leaf (9). It is transmitted by the leafhoppers Empoasca devastans Dis. and Eutettix phycitis Dis. Hill (7) states that "It seems likely that the diseases known as 'big bud' in Australia, 'stolbur' and 'montar' in the U.S.S.R. and 'little leaf' in South India, are all due to the same virus or to very closely related viruses." The symptoms produced by big bud of tomato and by false blossom in tomato in the United States suggest these diseases also belong in the stolbur group. They probably are produced by closely related strains of one virus. Several plants on which big bud in Australia have been found occurring naturally or to which it has been transmitted have been shown to be susceptible to false-blossom virus. These include tomato, tobacco, parsley, parsnip, cultivated carrot, celery, *Phlox Drummondii* Hook., and *Calendula officinalis* L. Both viruses are known to affect species in the genera *Petunia*, *Centaurea*, *Gaillardia*, and *Dianthus*. Thus two of the viruses in the group show similarities in host ranges as well as in symptoms produced. False blossom causes woodiness in fruits and stems similar to that described by Michailowa (20) for stolbur in tomato. These two viruses produce histological changes that are closely parallel. It is also interesting to note that of the four leafhoppers known to spread diseases of this type, three, *Euscelis striatulus*, *Eutettix phycitis*, and *Thamnotettix argentata*, are in closely related genera, while the fourth, *Empoasca devastans*, is in a closely related division of the subfamily Jassinae.

False blossom is the first disease in the group to be cured by heat. If the other diseases are as closely related as is believed they also should respond to heat treatments. Although false blossom has been cured only in the periwinkle and the cranberry it seems likely that it might be cured in many other species. Whether the heat cure described in this paper will be of any practical value to cranberry growers is not known. If some method could be found of warming up the water in flooded cranberry bogs it might be possible to cure the tops of diseased plants at least.

It is not known why some cranberry plants were cured by treatment at 43° C. for 7 days, while others were not. However, this was the mildest treatment that proved effective and it is possible that the plants which retained virus had somewhat thicker stems or were rooted more deeply than those that were cured. Judging by results of the experiments reported in this paper treatments at 42° or 43° C. for about 8 days are to be recommended for cure of cranberry plants. False-blossom virus proved to be somewhat less sensitive to heat than the viruses of aster yellows (14), peach yellows (13), or potato witches'-broom (18).

SUMMARY

- 1. Cranberry false-blossom virus was transmitted by dodder, Cuscuta campestris, from cranberry to 28 different species of plants belonging in 10 different families. It was transmitted by dodder from Vinca rosea and tomato to cranberry plants. It was retained by dodder growing on healthy plants over a period of two years, and apparently multiplies in this vector.
- 2. False-blossom virus also was transmitted to *Vinca rosea*, Turkish tobacco, tomato, potato, *Nicotiana glutinosa*, and *N. rustica* by grafting.
- 3. It was not transmitted mechanically by means of juice, by the aster leafhopper, *Macrosteles divisus*, or through dodder seeds produced by the parasite while growing on a diseased tomato plant.
- 4. In all the species to which it was taken false blossom produced yellows type symptoms. In many it caused gigantism in flowering and fruiting organs; it caused sterility in the tomato.
 - 5. False blossom in periwinkle and cranberry was cured by heat treat-



Treatments at 42° or 43° C. for about 8 days are recommended ments. for cure of cranberry plants.

6. It is suggested that false blossom may be closely related to big bud of tomato in Australia and the United States, to stolbur of tomato in the U.S.S.R., and to little leaf of eggplant in South India.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.

PRINCETON, NEW JERSEY.

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A LEAF SPOT OF COWPEA AND SOYBEAN CAUSED BY AN UNDESCRIBED SPECIES OF HELMINTHOSPORIUM

LINDSAY S. OLIVE, 1 DOUGLAS C. BAIN, 2 AND C. L. LEFEBVRE3

(Accepted for publication May 25, 1945)

On August 14, 1944, several leaves of Vigna sinensis (L.) Endl. with leaf spots of a type not heretofore observed on this plant were collected from a severely infected field near La Place, Louisiana, and examined for pathogens. The only organism constantly associated with these spots was a species of Helminthosporium which does not appear to have been previously described. At La Place, the fungus was causing a great deal of damage to several varieties of cowpea over an area of 50 acres. It was not found by us in any other locality in the State, nor on any plant other than the cowpea in that area.

Later, we observed a note in the Plant Disease Reporter (Vol. 28: 970. October 1, 1944) to the effect that an unidentified species of Helminthosporium had been found on cowpea leaves by the Bureau of Entomology and Plant Quarantine. At our request, Dr. D. P. Limber generously sent us specimens of this material. Three collections, all made by A. W. Blizzard, were examined. Two of the collections—one from Burton, South Carolina, the other from Elizabeth City, North Carolina-were found to be identical with our fungus. A third collection from Seabrook, South Carolina, is still another species which we do not find listed for cowpea or any other leguminous host.

On looking over some diseased soybean specimens collected in Florida during 1943, a few leaves of Mamredo and Edsoy varieties collected in August had several leaf spots caused by a fungus which appeared to be identical with the one on cowpea. A culture of the fungus, made in August, 1943, was found to be still viable.

The only species of Helminthosporium reported on Vigna sinensis up to the present time is H. molle B. & C., which is entirely different from the species described in this paper. After a search through the literature, in which particular attention was given to all descriptions of species on leguminous hosts, we were unable to find any description of the fungus studied by us. Moreover, no previous report of a Helminthosporium on soybean was found.

The finding of this fungus primarily in endemic areas near ports of entry would seem to indicate that it has been recently introduced into the United States from some other country.

Pathologist, Emergency Plant Disease Prevention, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland.
 Pathologist, Emergency Plant Disease Prevention, Agricultural Experiment Station,

Baton Rouge, Louisiana.

³ Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland.

DESCRIPTION OF THE LEAF SPOT

This disease, to which we are giving the common name of target spot, is chiefly one of the foliage of cowpeas, where it first appears as numerous reddish-purple dots which gradually enlarge into conspicuous brown circular areas, often with wavy margins (Fig. 1, A). Mature spots almost always have an outstanding zonation, consisting of a varying number of reddish-brown rings against a lighter brown background (Fig. 1, B). Each spot generally has a small reddish-brown dot in the center. Several spots may coalesce to produce more extensive brownish areas on the leaf, but each one maintains its own identity by virtue of its conspicuous zonation and the

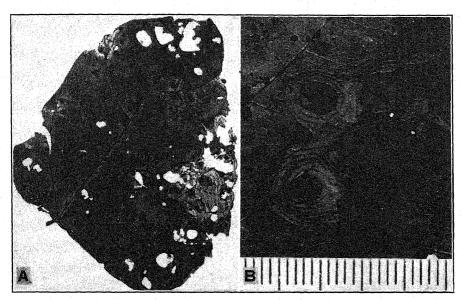


Fig. 1. Cowpea leaves showing natural infection with Helminthosporium vignae sp. nov. A. Leaflet with characteristic spots and shot-hole effect. B. Enlarged spots, showing concentric zonation.

central dot. The spots show on both surfaces of the leaf, but are more conspicuous on the upper surface. Eventually the dead brownish tissue may rupture and fall out completely, leaving holes in the leaf and imparting to the leaf an appearance of having been eaten out by insects (Fig. 1, A). Yellowing of the entire leaf and defoliation occur in severest cases. Most of our leaf specimens from the field have from 2 or 3 to an indefinite number (50 or more) of zoned spots and a larger number of small, purplish, undeveloped spots. The majority of zoned spots are 3 to 10 mm. in diameter, but some range up to 2 centimeters.

On diseased soybean from the field, a few less conspicuous brown spots caused by the fungus were found scattered over a few of the leaves. These spots did not have the zonation so characteristic of those on cowpeas. Damage to the soybean by this fungus does not seem to be serious.

STEM INFECTIONS

Later in the season it was observed that the fungus was capable of producing numerous reddish-purple spots and streaks of various sizes on mature stems and petioles of cowpeas already made unhealthy by severe foliage infection (Fig. 2). The *Helminthosporium* was found sporulating on some

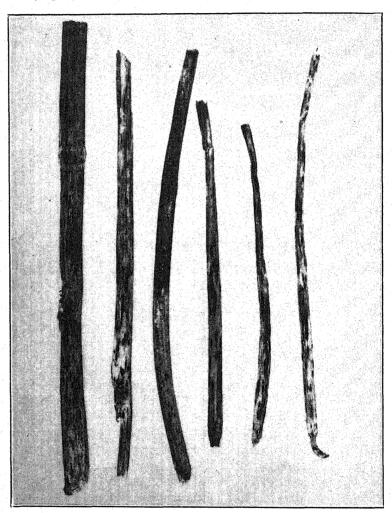


Fig. 2. Stems and petioles of cowpeas naturally infected with *Helminthosporium vignae* sp. nov. Natural size.

of these spots. On the other hand, we obtained almost no stem infection from inoculations of young cowpea plants in the greenhouse, and no visible damage resulted from the few small and rather superficial purplish spots which did appear on the stems. Thus it appears that only old stems of unhealthy cowpeas are likely to be attacked with any severity by the fungus.

Other fungi were generally associated with the purple stem spots of field specimens and were undoubtedly partly responsible for the severity of the stem infections.

No spotting of soybean stems by this fungus has been observed.

DESCRIPTION OF THE FUNGUS

When diseased cowpea leaves are brought in from the field and examined under low power, numerous elongate, dark brown conidiophores may generally be seen on both surfaces of the leaf spot. Conidiophores tend to be more abundant on the reddish-brown rings than between them. They occur either singly or in groups of 2 to 6.

Under high-power magnification the conidiophores are 1–20-septate and variable in length, measuring 6–11 \times 44–380 μ . The majority are 3–5-septate and $8\times125–200~\mu$. They arise from swollen basal cells and produce conidia singly or in chains at their tips (Fig. 3, B and C). Rarely a condiophore may grow beyond the point where the first conidium was formed to produce a second one (Fig. 3, D).

When the cowpea and soybean leaves are first brought into the laboratory for examination, the conidia present on the leaf spots are typically brownish, elongate, broadest at the base and very perceptibly tapered towards the apex (Fig. 3, A). They measure 8–19 \times 40–270 μ and are 3–20-septate, the average being about 17 \times 120 μ and 10-septate. They may be straight or curved, the latter characteristic applying to most of the longer ones. Some are long-cylindrical and of almost the same diameter throughout (Fig. 3, D). Their walls are thickened in the manner characteristic for the genus.

When diseased leaves are placed in a moist chamber, the conidiophores tend to be slightly longer, but do not vary significantly from the description given. However, few of the conidia formed in the moist chamber have the broad base and tapering apex which are typical of those produced in the field. They are generally straight or somewhat curved, long and slender, and usually taper very gradually, if at all, towards the apex (Fig. 3, B and E). They measure 7–11 \times 40–306 μ and are 1–20-septate, averaging 10×150 –250 μ and 10–15-septate. They appear in chains of 2–5. This tendency towards catenulation has been frequently observed in the field early in the morning while the dew still covers the plants. It will be described more fully under the discussion of cultural characteristics.

The conidium generally becomes multiseptate before it is shed. On germination it produces a germ tube at each end (Fig. 3, E). At the basal end there is a hilum, containing a centrally located pore through which the germ tube passes (Fig. 3, A, E, and F). The other germ tube passes directly through the apex of the conidium where the wall is very thin (Fig. 3, E). A few conidia have been seen to produce an additional germ tube from an intermediate segment, but this is rare.

When diseased stems are placed in a moist chamber numerous conidiophores bearing conidia appear on them. Measurements fall within those

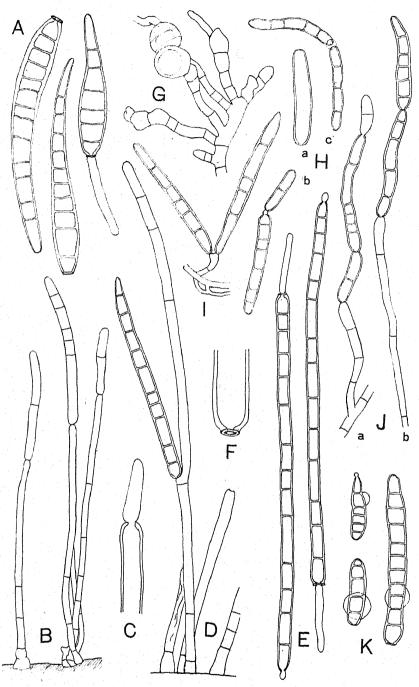


Fig. 3. Helminthosporium vignae sp. nov. A-E. Conidiophores and conidia from diseased cowpea leaves. F. Hilum end of conidium. G. Chlamydospores from agar culture. H-K. Conidia and conidiophores from agar culture. All figures \times 340, except B, \times 225, and C and F, \times 710.

already given for the fungus on the leaf spots: most of the condia are 8–11 \times 86–196 μ and are 4–7-septate, while the conidiophores reach their maximum length here, measuring 6–8 \times 34–750 μ .

No definite morphological differences between the fungus on cowpea leaves and stems and the one on soybean leaves have been observed. Certain physiological differences have been determined, however, and these will be described later in connection with inoculation results.

CULTURAL CHARACTERISTICS

Single-spore cultures of the fungus were made and its growth rate and degree of sporulation tested on potato-dextrose, corn-meal, prune, malt, and Czapek's-solution agars. It grew and fruited on all of these, but the best production of conidia occurred on potato-dextrose and on Czapek's-solution agar, the poorest on malt agar. In most cases the rate of growth is moderate and conidia begin to appear about 5 to 6 days after the culture is started. The hyphae at first form a white flocculent mass, which later becomes dark gray and forms an olivaceous black mat at the surface of the agar. This dark discoloration eventually extends throughout the agar medium. Hyphae and conidia at first appear nearly hyaline under the microscope, but soon become dusky brown.

In culture the conidia are typically borne in chains of 2–5, or possibly more (Fig. 3, J). They appear to be produced acropetally only; that is, the first conidium produces at its apex a secondary conidium, which in turn may give rise to a third in the same way and so on. Frequently as many as 3 are found in a row before any of them have matured. Often they begin to germinate before they separate from the chain, in which case the developing germ tubes impart to the conidia the appearance of being connected by little intercalary plugs (Fig. 3, H, b). This same tendency towards catenulation was also observed by Drechsler⁴ in *Helminthosporium catenarium* Drechs. and by Berg⁵ in *H. papulosum* Berg.

Frequently hyaline vesicles, probably gelatinous in nature but with function unknown, are found in various positions on the conidia. A vesicle is sometimes at the point of contact of two conidia in a chain (Fig. 3, H, c); at other times it is around some of the intermediate segments (Fig. 3, K). Similar structures were also reported by Berg for Helminthosporium papulosum. Our fungus has several characteristics which seem to ally it with the species studied by Berg, the catenulate nature of the conidia in a moist environment and the occurrence of vesicles on them in culture being two of the most important similarities. The vesicles were not observed on conidia produced on leaf and stem spots in nature.

Rarely, a conidiophore produced in culture bears two conidia at its tip (Fig. 3, I). While most of the conidia are 4-8-septate, they may range from

Berg, A. Black pox and other apple-bark diseases commonly known as a West Virginia Agr. Exp. Stat. Bull. 260. 1934.

<sup>Drechsler, C. Some graminicolous species of Helminthosporium: I. Jour. Agr. Res. [U. S.] 24: 670. 1923.
Berg, A. Black pox and other apple-bark diseases commonly known as measles.</sup>

unicellular to 15-septate and measure 7–12 \times 26–204 μ in culture on potato-dextrose or Czapek's-solution agar (Fig. 3, H–K). Some are broader at the base and taper conspicuously towards the tip, but the majority are cylindrical and about the same width throughout. They vary from straight to conspicuously curved. Conidiophores in culture are mostly 1–4-septate and extremely variable in size, measuring 4.5–8 \times 26–440 μ .

Soon after the culture becomes well established, numerous chlamydospores generally appear. They are most abundant in older cultures and cultures made by repeated transfers. They are hyaline, terminal or intercalary, and measure $14-20\times16-30~\mu$.

With respect to cultural characteristics, no essential differences were observed between the fungus isolated from cowpea leaves and the one from soybean leaves.

INOCULATION EXPERIMENTS

Cultures of the fungus isolated from cowpea leaves collected in Louisiana and from leaf spots of Mamredo soybean collected in Florida, were used in making inoculations. These inoculations consisted of spraying the plants with an aqueous suspension of spores and hyphae of the fungus. The pathogenicity of the fungus from cowpea was tested on four varieties of cowpea (Blackeye, Early Buff, Early Silver Crowder, and Iron) and on six varieties of soybean (Nanda, Avoyelles, Otootan, Biloxi, Seminole, and Mamredo). All varieties of cowpea showed signs of infection within 3 days following inoculation. In 6 days the results were outstanding. Many conspicuous purple spots appeared on the leaves, followed by a yellowing of the more severely infected leaves. Considerable defoliation was the final result. The variety of cowpea most severely attacked was Iron; the least injured was Blackeye. Damage to Early Buff and Early Silver Crowder was severe.

Leaf spots appearing on inoculated cowpeas eventually turned brown, but each retained a purple border and a purple dot in the center. These spots did not have the concentric zonation found on diseased leaves collected in the field (Fig. 4, A). The fungus was readily recovered from the spots induced by inoculation.

All 6 varieties of soybean showed slight infection in the form of small, scattered, purplish spots which appeared about 3 days after inoculation and had little or no tendency to enlarge. These spots often had a light yellow discoloration around the border. They became brown, but remained small and without concentric zonation. Since the infection on soybean was light, no defoliation resulted. The fungus was recovered from leaf spots on these inoculated soybeans.

In spite of its morphological similarity to the fungus isolated from cowpea, the fungus isolated from Mamredo soybean did not give the same results on inoculation. The Iron cowpea, which was so heavily spotted by the former, was only slightly infected when inoculated with the latter. Few to many small purple specks appeared on the leaves, but these tiny spots

showed little tendency to enlarge and caused little or no permanent damage to the plants. When several Biloxi and Mamredo soybean plants were inoculated with the fungus, numerous, small, reddish-purple spots developed (Fig. 4, B). These spots showed little tendency to enlarge and eventually turned brown. They were very similar to those produced by the fungus from cowpea leaves. No defoliation occurred and damage to the plants was

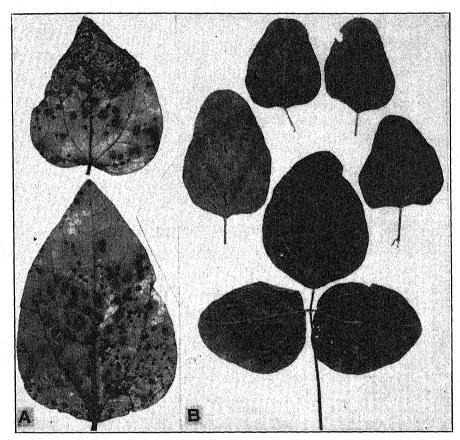


Fig. 4. Results of artificial inoculation with *Helminthosporium vignae* sp. nov., showing spots on leaves of cowpea (A) and soybean (B).

almost negligible. The fungus was recovered from leaf spots on inoculated soybeans and from a few spots on inoculated cowpeas.

It is apparent that two parasitic races of the *Helminthosporium* have been involved in these investigations. Race 1, isolated from cowpea leaves, is capable of causing severe infection of cowpeas and a light spotting of soybeans. Race 2, isolated from leaves of Mamredo soybean and morphologically similar on the host tissue as well as in culture to Race 1, produces a light spotting of soybean leaves and few to many very small spots on the cowpea, with little or no damage resulting to either host.

It was discovered that, after 3 or 4 transfers of Race 1 had been made. this race of the fungus lost its pathogenicity and failed to produce any infection on either cowpea or soybean. Very often conidia ceased to appear after repeated transfers of the fungus had been made. However, loss of pathogenicity does not appear necessarily to be related to a lag in conidial production, since some old cultures which were still producing conidia had lost their virulence. The most effective inoculum was made from cultures grown directly from single conidia taken from the diseased leaf, without any intervening transfers. Race 2 seemed to retain its pathogenicity better. All cultures of it used in inoculations came originally from a culture which had been isolated in August, 1943. It produced conidia abundantly and proved to be as pathogenic to soybeans as Race 1 after we rejuvenated it by growing it on oats and then placing the oats on agar.

The only sign of stem infection in any of the inoculation experiments was the appearance of a few rather superficial purplish spots on the stems of some of the cowpeas inoculated with Race 1. These spots became less conspicuous as growth of the plants continued, and no damage was caused by them. The fungus was not found sporulating on any of the leaf spots resulting from inoculations until diseased leaves were placed in a moist chamber. Conidia produced on these leaves tended to be longer and less tapering than those already described (Fig. 3, E).

DIAGNOSIS6

HELMINTHOSPORIUM vignae Olive, sp. nov. Maculae in foliis Vignae primum punctiformes, parvae et purpureae, dein majores, brunneae, conspicuae, concentrice zonate, demum secedentes, in foliis Sojae minores, brunneolae, ezonatae; conidiophora amphigena, singula vel 2-6-caespitosa, fusca, 6-11 \times 44-380 (-490) μ , 1-20-septate, typica 8 \times 125-200 μ et 3-5-septata; conidia fusca, acrogena singulata vel catenulata oriunda, recta vel curvata, basi lata, apice insigniter attenuata, interdum anguste cylindrica, 8-19 \times 40-270 μ , 3-20septata, typice 15–18 × 100–180 μ et circa 10-septata.
In foliis caulibusque Vignae sinensis et foliis Sojae max in Carolina boreali et aus-

trali, Florida, et Louisiana.

Spots on leaves of cowpea beginning as small purple dots and enlarging to conspicuous concentrically zoned brown spots which are often deciduous; stems with purplish spots and streaks late in the season; spots on leaves of soybean smaller, brownish, not zoned. Conidiophores appearing on both surfaces of the leaf, singly or in groups of 2-6, dark brown, 6-11×44-380(-490) μ , 1-20-septate, typically 8×125 -200 μ and 3-5-septate; conidia brown, produced singly at the tips of the conidiophores, becoming catenulate under very moist conditions, straight or curved, with broad base and conspicuously tapering apex, sometim's varying to narrow cylindrical, 8-19 × 40-270 u, 3-20-septate, typically 15-18 $\times 100-180\,\mu$ and about 10-septate, germinating by means of two polar germ tubes, the basal one passing directly through a pore in the center of the hilum.

In culture on Czapek's-solution agar or potato-dextrose agar, conidia appearing within 5-6 days, and floculent mycelium, white at first, becoming deep gray. Conidiophores mostly 1-4-septate and very variable in size, 4.5-8 \times 26-440 μ ; conidia cylindrical or tapering towards apex, often curved, typically in chains of 2-5, measuring 7-12 \times 26-204 μ , 0-15-septate; chlamydospores becoming numerous in older cultures, hyaline, mostly around

On leaves, or on stems late in the season, of Vigna sinensis (L.) Endl., and on leaves of Soja max (L.) Piper; North Carolina, South Carolina, Florida, and Louisiana.

⁶ The authors wish to thank Miss Edith Cash for preparing the Latin diagnosis of the fungus described in this paper.

Specimens of diseased cowpeas and soybeans have been deposited in the Mycological Herbaria at the Beltsville Plant Industry Station and at Louisiana State University.

SUMMARY

A species of *Helminthosporium*, apparently heretofore undescribed, has been found to cause a severe leaf spotting of cowpeas, with stem infections taking place late in the season. This fungus also causes a light spotting of soybean leaves. Two parasitic races have been isolated. Race 1, isolated from cowpea leaves, causes a severe leaf spotting of cowpeas and a light spotting of soybeans; while Race 2, isolated from soybean leaves, produces a light spotting of soybean leaves and few to many small specks of little consequence on cowpea leaves.

PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND.

PARASITISM OF RHIZOCTONIA SOLANI FROM ALFALFA¹

OLIVER F. SMITH2

(Accepted for publication May 25, 1945)

In a previous publication (12) the writer described a root canker of alfalfa, which occurs in southwestern Arizona and southern California, and attributed its cause to *Rhizoctonia solani* Kühn. This fungus species is known to have a wide host range (2, 3, 9), and strains within the species have been recognized by several investigators (5, 6, 7, 10, 11). This publication reports results of pathogenicity tests on alfalfa with random isolates of *R. solani*, and tests of the pathogenicity on other crop plants, of the strain which causes alfalfa root canker.

METHODS AND MATERIALS

Plants were grown in galvanized iron cans, 7 inches in diameter and 11 inches deep. A sandy loam soil from Nevada was used with its natural microflora, except for the addition of a small amount of inoculum. Inoculum was prepared by growing *Rhizoctonia solani* for about two weeks on previously soaked and sterilized barley grain. When the plants had a good top growth, five holes, about ½ inch in diameter and 9 inches deep, were made in each can of soil and the holes filled with inoculum. The temperature of the soil was then kept at 29–30° C. Usually the plants were exposed to the organism for about 4 weeks before they were removed from the soil and their roots examined for lesions. In some cases, however, disease development was rapid enough to allow for root examination after a shorter period.

All results herein reported on alfalfa were obtained with the southern grown variety California Common.

PATHOGENICITY OF ISOLATES FROM ALFALFA TO ALFALFA

Isolates of *Rhizoctonia solani* were obtained from alfalfa grown in California and Nevada. Some of the isolates were from root lesions, some from fine transient roots (4), and some from young damped-off seedlings. A list of the isolates used and results obtained when California Common alfalfa was inoculated with them are in table 1.

Only certain isolates produce root lesions on alfalfa (Table 1). Isolates obtained from alfalfa grown in Nevada caused no root lesions, whereas isolates from root lesions on alfalfa grown in California, where the root canker is prevalent, caused root lesions. Since one isolate from a transient root of alfalfa, grown at Bard, California, did not produce root lesions, it is evident that isolates which cause root lesions and isolates which do not cause root lesions may be obtained from areas where the disease occurs.

¹ Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nevada Agricultural Experiment Station.

² Associate Pathologist, Division of Forage Crops and Diseases, U. S. Department of Agriculture.

PATHOGENICITY OF ISOLATES FROM OTHER PLANT SPECIES TO ALFALFA

To determine the pathogenicity of Rhizoctonia solani from other crop plants to alfalfa, isolates known to be pathogenic on certain plants were used, as well as some that had not been tested for pathogenicity. Isolates were obtained from sclerotia on potato tubers, and from underground lesions on potato stems. Others were obtained through the courtesy of W. J. Cherewick, 3 John E. Kotila, 4 and D. C. Neal. 5 Two isolates were furnished by Dr. Cherewick; one P-66, reported to be pathogenic on potatoes, and the

TABLE 1.—List of isolates of Rhizoctonia solani and results obtained from greenhouse inoculation to alfalfa. All isolates obtained from alfalfa

Isolate No.	Place where plant was grown	Part of alfalfa plant from which isolated	Inoculation results ^a
92	Minden, Nevada	Lesion below ground on stem of	
		young seedling	0/28
122	do		0/27
115	do	Root lesion	0/21
96	Reno, Nevada	Damped-off seedling in green-	
	arovada	house	0/28
119	đo	do	0/25
120	do	đo	0/23
121	do	do	0/22
98	Bard, Calif.	Transient root	0/27
102	do	Root lesion	28/28
103	do	do	4/26
105	do	do	21/21
127	El Centro, Calif.	do	$\frac{21}{21}$
128	do	do	20/23
129	do	do	$\frac{20/20}{21/30}$
130			$\frac{21}{30}$
	do	do	
$131 \\ 132$	do do	đo đo	$\frac{30/40}{23/26}$

a Numerator = number of alfalfa plants with root lesions. Denominator = number of alfalfa plants inoculated.

other, S.C.R.I., reported as causing a crown rot and seedling damping-off of sweetclover and alfalfa (1). Three isolates (R-167, R-216, and R-380) were furnished by Dr. Kotila; R-167 and R-216 are reported as causing damping-off and root rot of sugar beets, and R-380 as causing damping-off and foliage blight of sugar beets. One isolate, pathogenic on cotton leaves (8), was furnished by Dr. Neal. The results from inoculation tests on alfalfa plants with these isolates of Rhizoctonia solani are in table 2.

In only one instance (R-216) was Rhizoctonia solani from other crop plants capable of causing root lesions on alfalfa (Table 2). In this case 4 of 40 roots inoculated with R-216 had root lesions; one root had 3 lesions, and three roots had one lesion each. The test was repeated with 50 alfalfa

³ Agricultural Assistant, Dominion Laboratory of Plant Pathology, Winnipeg, Mani-

toba, Canada.

4 Pathologist, Division of Sugar Plant Investigation, U. S. Department of Agricul-

⁵ Senior Pathologist, Cotton Disease Investigation, U. S. Department of Agriculture, University of Louisiana, Baton Rouge, Louisiana.

TABLE 2.—Results obtained from greenhouse inoculation tests to alfalfa with isolates of Rhizoctonia solani from other plant species. Inoculated June 1, observed July 22, 1944

Isolate no.	Host plant	Part of plant from which isolated	By whom isolated	Inoculation results:
R-116	Potato	Sclerotium on potato tuber	O. F. Smith	0/25
R-117 R-126	do do	do Underground lesion on potato stem	do do	0/21 0/38
P-66 S.C.R.I. R-167 R-216 R-380 R-134	do Sweetclover Sugar beet do do Cotton	Root	W. J. Cherewick do John E. Kotila do do D. C. Neal	0/50 0/58 0/27 4/40 0/32 0/51

a Numerator = number of alfalfa plants with root lesions. Denominator = number of alfalfa plants exposed to organism.

plants, using isolate R-216 of R. solani, and 8 of 50 plants inoculated had one or more lesions. Alfalfa plants inoculated at the same time with isolate 102, obtained from a root lesion on alfalfa at Bard, California, averaged 29.4 lesions per main root for 25 plants. Thus R-216 can be considered as being only very weakly pathogenic on alfalfa roots under the conditions of these experiments.

PATHOGENICITY OF RHIZOCTONIA SOLANI, ISOLATE 102, ON PLANTS OTHER THAN ALFALFA

Isolate 102 of Rhizoctonia solani is very pathogenic on alfalfa roots (Table 1). It seemed advisable to determine if this particular isolate is pathogenic on other crop plants, some of which are grown in southern California and southwestern Arizona where Rhizoctonia root canker is prevalent

TABLE 3.—Results obtained in 1944 by inoculating several plant species with isolate 102 of Rhizoctonia solani from alfalfa

Plant	I	Date	Inoculation
	Inoc.	Examined	resultsa
Canada field pea, Pisum sp. Bard vetch, Vicia calcarata Desf. Berseem clover, Trifolium alexandrinum L. Guar, Cyamopsis tetragonolobus Taub. (C.	Jan. 1	Jan. 14	20/41
	do	do	35/35
	Mar. 17	Apr. 13	7/67
psoraloides DC.) Hubam sweetclover, Melilotus alba Desr. Sour clover, M. indica All. Madrid Evergreen sweetclover, M. alba Desr. Cumberland red clover, Trifolium pratense L. Alfalfa, Medicago sativa L.	do	do	6/25
	do	do	19/40
	do	do	18/65
	June 1	July 22	32/34
	do	do	10/10
	do	do	33/33

a Numerator = number of plants with root lesions.

Denominator = number of plants with 1000 lesions.

Denominator = number of plants exposed to organism.

Control plants of all species were treated exactly like inoculated plants except that no organism was growing on the barley grain added to the soil. All control plants

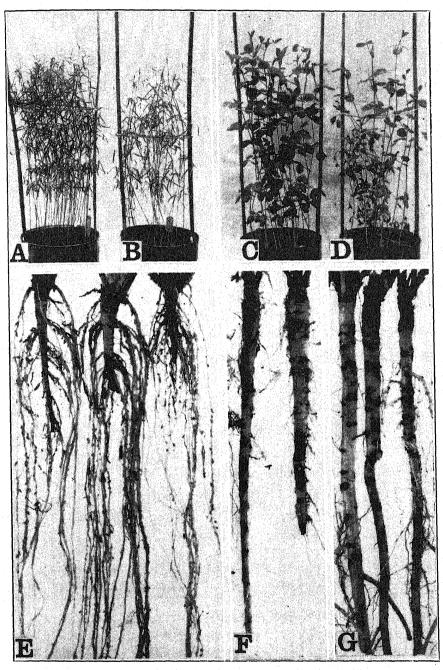


Fig. 1. Effect of *Rhizoctonia solani* on several plant species at a soil temperature of 29°-30° C. A, Bard vetch, not inoculated; B, Bard vetch, inoculated; C, Canada field pea, not inoculated; D, Canada field pea, inoculated; E, Cumberland red clover, inoculated; F, Madrid sweetclover, inoculated; G, Alfalfa, inoculated. A through D photographed two weeks after inoculation, E through G photographed approximately seven weeks after inoculation.

on alfalfa. Results from inoculation tests with isolate 102 on several plant species are in table 3.

Isolate 102 of Rhizoctonia solani is pathogenic on several plant species. but it is more pathogenic on some than on others. However, the effects of the organism on the different species are not alike. On alfalfa (Fig. 1, G). Madrid sweetclover (Fig. 1, F), and guar, the organism causes characteristic, circular, dark lesions on the main tap root and large lateral roots, but no marked symptoms on top growth until the disease has reached an advanced stage of development, at which time badly infected plants are somewhat stunted and some are killed. On Canada field peas, the organism invades the stem at the cotyledonary node and top symptoms no not appear until the stems are practically rotted off, then the plants wilt and dry rapidly (Fig. 1, D). No lesions were observed on the roots of Canada field pea. On Bard vetch the entire root system seemed to be rotted and partially destroyed, and the plants considerably weakened within two weeks (Fig. 1, B). On red clover the main tap root was rotted off and an increased number of lateral roots was produced on the tap root above the place where it had been severed (Fig. 1, E). On sour clover, very few single lesions were produced, but the main tap root and most of the fine feeder roots of infected plants were badly rotted. Berseem clover was apparently quite resistant as there were very few lesions on roots of affected plants.

There was no indication of host specialization in the strain of *Rhizoctonia* solani which causes alfalfa root canker. In inoculation tests it has been as pathogenic on other crop plants as on alfalfa. It is a very pathogenic strain capable of parasitizing several plant species.

SUMMARY

California Common variety of alfalfa was inoculated with isolates of *Rhizoctonia solani* from other plant species, and other plant species were inoculated with a strain of *R. solani* which causes a root canker of alfalfa.

Isolates from root cankers of alfalfa caused abundant root lesions when reinoculated to alfalfa. Isolates from other plant species produced no lesions on alfalfa roots, except isolate R-216 which was only weakly pathogenic and produced only a very few lesions on alfalfa.

Isolate 102, which causes root lesions on alfalfa, was pathogenic on roots of Bard vetch, Berseem clover, guar, Hubam sweetclover, sour clover, Madrid Evergreen sweetclover, and Cumberland red clover. It also was pathogenic on stems of Canada field pea.

NEVADA AGRICULTURAL EXPERIMENT STATION AND

DIVISION OF FORAGE CROPS AND DISEASES, U. S. DEPARTMENT OF AGRICULTURE.

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STUDIES ON ASCOCHYTA IMPERFECTA, A SEED- AND SOIL-BORNE PARASITE OF ALFALFA¹

M. W. CORMACK²

(Accepted for publication June 2, 1945)

Ascochyta imperfecta Peck is best known as the cause of black stem of alfalfa. This disease, first described in New York in 1908 (16), is now widely distributed in many alfalfa-growing areas and is of considerable economic importance in certain regions (7, 13, 14, 17). Most of the damage occurs on varieties of Medicago sativa, but the pathogen can attack other species of Medicago, and also Medilotus and Trifolium (2). Non-leguminous hosts have not been reported.

Ascochyta imperfecta was officially identified as the cause of black stem of alfalfa throughout Canada in 1938 (3), when it was also found associated with a root rot of alfalfa and other legumes in Alberta. Subsequent studies have revealed that this fungus is commonly carried on the seed of alfalfa, as well as present in the soil of alfalfa fields, and that it can attack the seedlings under certain conditions. These previously unreported findings, and additional data obtained on the host range and life history of A. imperfecta, are presented in this paper.

OCCURRENCE OF ASCOCHYTA IMPERFECTA IN ALBERTA Black Stem and Leaf Spot

Ascochyta imperfecta occurs commonly throughout Alberta on the stems and leaves of alfalfa. Infection is favored by cool, wet periods during the spring and fall. Although the damage is sometimes severe in certain seasons in isolated cases, it is not of major importance in Alberta. Actual killing of the plants, such as occurs in Kentucky (7), has not been observed. The most damage is caused by infection of the young shoots early in the season. Blackening of the stems occurs progressively and is usually not severe until late in the season in stands left for seed. Observational evidence indicates that severe infection of the flowering portion of the stem reduces the yield of seed. In Alberta, infection of the leaves is often more severe than that of the stems, and considerable defoliation may occur before the hay crop is cut.

Since the symptoms of black stem and leaf spot caused by Ascochyta imperfecta have been fully described by other workers (7, 13, 14, 17), only a few special features will be mentioned here. Stem lesioning ranges from slight spotting to complete discoloration (Fig. 1, A), and varies from light brown to deep black. It is often confined mainly to one side of the stem. When conditions are favorable, infection progresses up the stem to the racemes, even to the seed pods (Fig. 1, B, C). The pycnidia

¹ Contribution No. 815 from the Division of Botany and Plant Pathology, Science Service, Dept. of Agriculture, Canada.

² Assistant Plant Pathologist.

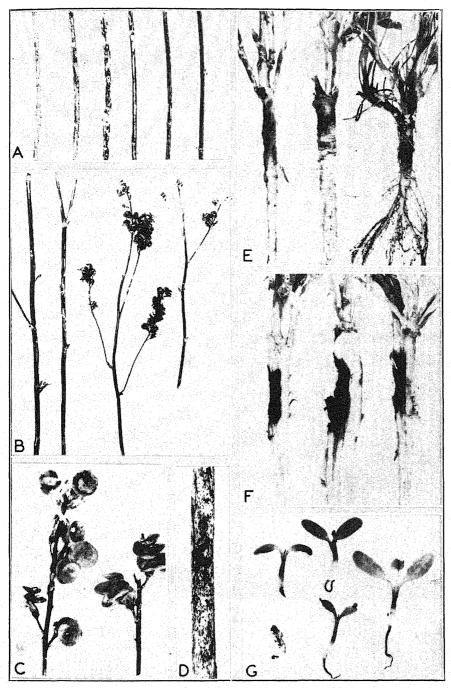


Fig. 1. Symptoms produced by Ascochyta imperfecta. A. Alfalfa stems infected to varying degrees. B. Entire alfalfa stem severely infected. C. Infected rachis and seed pods of alfalfa. D. Overwintered alfalfa stem with pycnidia. E. Affected roots of alfalfa, sweet clover, and red clover. F. Extent of rotting in roots of alfalfa, sweet clover, and red clover. G. Alfalfa seedlings from naturally infected seed.

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of A. imperfecta are often absent or only sparingly borne on infected stems during the growing season. When present, they are most abundant in slightly dried, bleached areas, and are relatively scarce in the darker or blackened portions of the stem. They increase greatly in number after growth ceases in the fall and are usually very numerous on the dead, overwintered stems (Fig. 1, D). The only other disease of alfalfa which might be confused with black stem is bacterial stem blight caused by Pseudomonas medicaginis Sackett. The latter, however, occurs very rarely in Alberta and can be distinguished from black stem by the lighter colored lesions, usually covered with bacterial exudate.

The leaf symptoms produced by Ascochyta imperfecta are extremely variable but usually appear as irregular, dark-brown spots. The leaf spot caused by Pseudopeziza medicaginis (Lib.) Sacc. sometimes occurs on the same plants but can be distinguished by the smaller, circular lesions with raised central discs. A. imperfecta rarely produces mature pycnidia on the leaves. When infection is severe the spots coalesce and the leaves soon turn yellow and drop.

Ascochyta imperfecta was readily isolated from typical lesions on the stems and leaves of Siberian yellow-flowered alfalfa (Medicago falcata), as well as those of several varieties of M. sativa. A few isolates of A. imperfecta were also obtained from blackened stems of sweet clover (Melilotus alba and M. officinalis), but Ascochyta lethalis Ell. & Barth. was predominant on this host. Black-stem lesions on red clover (Trifolium pratense) and alsike clover (T. hybridum) yielded only unidentified species of Ascochyta and Phoma. Black-stem and leaf-spot symptoms were also fairly common on various noncultivated legumes. A. imperfecta was isolated occasionally from diseased stems of Vicia americana and from leaves of Lathyrus spp., but unidentified species of Ascochyta predominated on these and other wild hosts.

Isolations from Roots

Ascochyta imperfecta was isolated from many diseased samples of alfalfa roots collected in various parts of Alberta, and from specimens sent from Saskatchewan. A few isolates were obtained from rotted roots of sweet clover. The fungus grew readily from diseased tissues picked out aseptically or surface-sterilized prior to plating. Usually it occurred in lesions with other root-rotting pathogens, especially Cylindrocarpon Ehrenbergi (4) and the low-temperature basidiomycete (5), and was detected only by isolation.

Symptoms of root attack typical of Ascochyta imperfecta were observed only in the relatively few cases where no other pathogen was present. The slightly sunken, dark-brown lesions usually occurred near the crown and rarely extended more than half way through the main root. Apparently infection occurred at the site of branch roots or wounds. The cracked or shredded tissues of these lesions sometimes contained a few pycnidia. Although the symptoms differ, this root rot is in some respects analogous

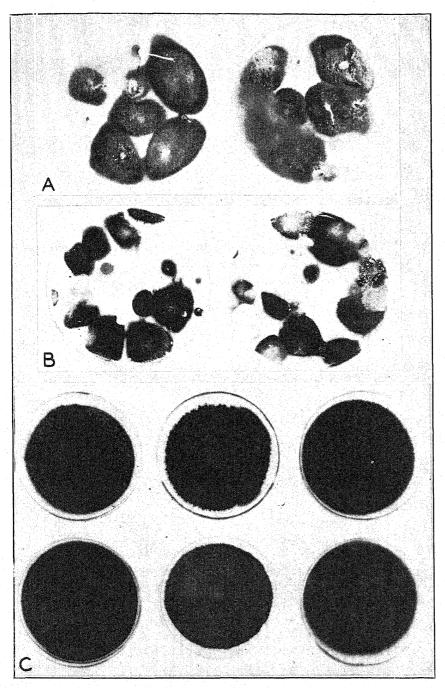


Fig. 2. A. Ascochyta imperfecta (dark-colored colonies) from naturally infected alfalfa seeds. B. Colonies in soil dilution plates from samples obtained from alfalfa fields. C. Ten-day-old cultures of 6 different isolates on potato-dextrose agar.

to the Stagonospora root rot of forage legumes described by Jones and Weimer (9).

Isolations from Seed

The fairly common occurrence of lesions on the racemes and seed pods of alfalfa suggested that the pathogen might be seed-borne. This was confirmed in seed isolation studies. The method used by Muskett and Malone (11) for the examination of flax seed proved most satisfactory. In most of the tests, 100 dry, untreated seeds of each sample were plated out on potato-dextrose agar. Surface disinfection was not practised, since it usually destroyed Ascochyta imperfecta and other fungi on the seed. After an incubation period of one week the characteristic, dark-colored colonies of A. imperfecta were easily recognized (Fig. 2, A), even when partly overgrown by Alternaria spp. or other saprophytic fungi. Seeds from all samples were also germinated on blotters or in soil, and isolations were made from the diseased seedlings.

TABLE 1.—Prevalence of Ascochyta imperfecta and other pathogens in samples of alfalfa seed from different sources (1941 and 1942 crops)

Source of seed	No. of	Number of samples infecteda						
	samples	Ascochyta	Stagonospora	Sclerotinia				
	examined	imperfecta	meliloti	sclerotiorum				
Northern Alberta Central Alberta Southern Alberta Other Provinces	35 11 39 12	18 7 19 5	3 1 3 0	3 0 0				
Total number	97	49	7	3				
Percentage		50.5	7.2	3.1				

a Containing one per cent or more of infected seed or sclerotia.

Isolations were made from seed harvested from 10 alfalfa stands having known amounts of black stem, and a direct relationship was found between seed infection and plant infection. The pathogen was isolated from 30 to 50 per cent of the seeds from fields in which infection was severe on the upper portions of the stems. When plant infection was slight, or was confined to the lower portion of the stems, from 90 to 100 per cent of the seeds escaped infection. It appears that seed infection occurs directly through the seed pods of severely diseased plants, and, to a lesser extent, by contamination during threshing and other operations.

The prevalence of Ascochyta imperfecta in alfalfa seed grown in 1941 and 1942 in various parts of Alberta was determined. The samples were obtained through the courtesy of the Plant Products Division, Production Service, Department of Agriculture, Calgary, Alberta, and were seed from foundation, elite, registered, and commercial stocks. Isolations were also made from a few samples received from the Provinces of British Columbia, Manitoba, Saskatchewan, and Ontario. A. imperfecta occurred in approximately one-half of the seed samples studied (Table 1). The pathogen was

isolated from seed obtained from all of the principal alfalfa-growing areas of Alberta, and also from at least one sample from each of the four other Provinces represented. These samples contained from 1 to 40 per cent of seeds infected with the fungus. Unfortunately, no information was available on the degree of infection in the stands from which they were harvested. The proportion of infected seed samples was approximately the same in the crop years of 1941 and 1942.

No association was found between the quality or appearance of the seed samples and their degree of contamination with Ascochyta imperfecta. Highly infected samples were found in all classes of seed examined. In tests with several samples containing 15 to 25 per cent of discolored, shrivelled, cracked, or green seeds, the pathogen was isolated as often from the sound, mature seeds as from the abnormal ones. It also occurred commonly on the hulls or other debris in poorly cleaned samples.

The results of dilution platings and microscopic examination of the sediment obtained by centrifuging samples of infected alfalfa seed strongly suggest that Ascochyta imperfecta persists mainly as mycelium on or in the outer portion of the seed coat. Spores were not observed in the sediment or washings from any sample. Moreover, pycnidia were not found on infected pods or seeds.

The only other pathogens found in alfalfa seed were Stagonospora meliloti (Lasch) Petrak (Leptosphaeria pratensis Sacc. & Briard), and Sclerotinia sclerotiorum (Lib.) de Bary. The sclerotia of the latter were mixed with the seed of a few samples. Neither of these pathogens was nearly as prevalent as Ascochyta imperfecta (Table 1). Also, no sample contained more than 2 per cent of seeds infected with S. meliloti. Alternaria spp. were most numerous among the various apparently saprophytic fungi isolated.

Ascochyta imperfecta was not found during the routine examination of several seed samples of sweet clover, red clover, and alsike clover. Stagonospora meliloti, previously reported as seed-borne by Jones (8), was found much more frequently in sweet-clover seed than in alfalfa seed.

Isolations from Soil

Samples of soil were taken in the fall from several alfalfa fields of varying age, cereal fields of known history, virgin prairie, and from virgin woods. They were taken one-half inch below the surface in all cases, and also at depths of 2 and 6 inches in the alfalfa fields. Soil dilutions were made, and plates of potato-dextrose agar poured in the usual manner. The colonies of A. imperfecta (Fig. 2, B) in each plate were counted after incubation for 7 days at room temperature.

Apparently Ascochyta imperfecta is much more prevalent in the soil of long-established stands of alfalfa than in the soil of those recently planted, and is most abundant at the one-half-inch depth (Table 2). It was isolated occasionally at the 2-inch depth, and, in the older stands,

TABLE 2.—Occurrence and distribution of Ascochyta imperfecta in certain cultivated and uncultivated soils in Alberta

Source of soil	Number	of samples	Ave col	erage numb onies per p	er of plate
Crop and history of field	Examined	Infested with A. imperfecta	0.5 inch depth	2 inch depth	6 inch depth
Alfalfa, 3 years old or older	10 3 5 2 2 2 10 3 3	10 3 5 0 0 0 0	9 4 4	0.5	0.1

at the 6-inch depth. In the cereal fields, it occurred fairly commonly the first year after alfalfa sod was plowed, but was not obtained the second and third year. It is significant that the pathogen was not obtained from the soil of the cereal rotations or from the samples taken from the virgin prairie, and woods.

PATHOGENICITY OF ASCOCHYTA IMPERFECTA ON ALFALFA AND OTHER LEGUMES $Pathogenicity\ on\ Stems\ and\ Leaves$

Representative isolates of Ascochyta imperfecta from the roots, stems, leaves, or seed of Medicago, Melilotus and Lathyrus, and from soil were tested for pathogenicity on the stems and leaves of alfalfa and other legumes. In greenhouse and field tests, vigorously-growing plants about three months old were sprayed or swabbed with a spore suspension of the pathogen (15). In the greenhouse, the inoculated and control plants were kept in a moist chamber for 3 days and were then placed under a bench until the symptoms developed. In the field, light frames covered with cheesecloth were placed over the plants for the entire period of incubation. A fairly high humidity was maintained within these cages by

TABLE 3.—Relative virulence of isolates of Ascochyta imperfecta on the stems of Medicago, Melilotus, and Trifolium. (Greenhouse and field tests.)

Isolates			 	f stem infec	ation o	
Source	Number	Medicago	 	elilotus	Trifolia	ım
Medicago, roots Medicago, stems Mcdicago, leaves Medicago, seed Medicago, seed Melilotus, roots Melilotus, stems Lathyrus, leaves Soil	tested 2 4 1 2 1 1 1 1	S-M S-M M M S-M S-M S-M	alba T-S S	officinalis p	S S S 	T T

a T-trace, S-slight, M-moderate.

constantly moistening the cheesecloth by means of cloth wicks leading into containers of water. After about 2 weeks the pathogen ceased to progress, and a final estimation was made of the degree of infection.

The typical symptoms of black stem and leaf spot were readily induced by the methods described, but infection was usually less severe than under natural conditions. Pycnidia seldom developed under conditions of artificial inoculation, but the pathogen was easily reisolated from the lesions on the stems and leaves.

On alfalfa stems, the isolates tested caused only slight to moderate infection (Table 3). The leaves, however, were slightly more susceptible. Four isolates, consisting of 2 from alfalfa stems, one from leaves of *Lathyrus* and one from the soil, caused moderate to severe defoliation of the plants.

A few of these isolates were also tested on other legumes (Table 3). A trace to slight infection developed on the stems and leaves of sweet clover, mainly in the vicinity of wounds. Some plants of Melilotus alba (variety Arctic) appeared to be slightly more susceptible than those of M. officinalis (variety Yellow Blossom). There was slight infection of the stems and leaves of red clover, and only a trace on alsike clover. Peas were not attacked by any of the isolates. These results are in general agreement with those reported by previous workers. Sprague (15) obtained slight infection of Melilotus spp., Trifolium pratense, and T. hybridum with Ascochyta imperfecta. Toovey, Waterston, and Brooks (17) found the pathogen could attack Medicago lupulina, Trifolium pratense, and Vicia sativa, but not T. repens, V. faba, or Pisum sativum. Medicago falcata and M. ruthenica were added to the host range by Peterson and Melchers (13).

With regard to varietal resistance in alfalfa, studies started in the field are not yet complete. However, other workers (7, 13, 17) have reported differences in varietal reaction, and Koepper (10) has isolated a selection of Ladak that is highly resistant to *Uromyces striatus* and *A. imperfecta*.

Pathogenicity on Roots

The pathogenicity of Ascochyta imperfecta to roots of alfalfa and other legumes was studied in the field in summer and winter tests. Plants about one year old were inoculated by placing cotton pads containing oathull-soil inoculum against the partially bared tap roots (4). Since preliminary experiments showed that wounding greatly facilitated root infection by this fungus, the roots were slightly cut prior to inoculation. The roots of the noninoculated control plants were treated similarly. The plants inoculated during the growing season were dug up in about 4 weeks, but those inoculated in the fall were not examined until the following spring. The degree of infection of each root was estimated and expressed in percentage by means of the disease rating previously described (4).

Disease symptoms from the fall inoculations of the roots of alfalfa did not appear until the soil thawed out in the spring, and then the rotting was slower than in comparable tests made during the summer. Apparently this fungus does not attack the roots at near-freezing soil temperatures, as is the case with certain low-temperature pathogens in Alberta (5). Only a slight to moderate amount of disease occurred at any time and the symptoms were similar to those previously described as occurring in nature (Fig. 1, E, F). No difficulty was experienced in reisolating the pathogen from the marginal tissues of the root lesions.

Certain isolates of the pathogen were more virulent than others in rotting the roots of alfalfa (Table 4), regardless of whether they were from the roots, stems, leaves, or seed of alfalfa, or from other hosts. That is to say, there was no evidence of specificity among the isolates in these respects.

Although the root-rot symptoms were similar on the various legumes (Fig. 1, E, F), Medicago sativa was usually more severely attacked than Melilotus alba (Table 4). The reaction of Medicago falcata, Melilotus officinalis, and Trifolium pratense to isolates from alfalfa roots was similar to that of M. alba. T. hybridum had a slightly lower disease rating.

Pathogenicity on Seedlings

Alfalfa seedlings were readily parasitized by Ascochyta imperfecta when naturally infected seeds were germinated on agar or blotting paper. The fungus grew out rapidly and either inhibited germination or attacked and rotted the young cotyledonary shoots. Healthy seedlings in contact with the diseased ones were also killed. Less seedling blight developed when the seeds were germinated under more natural conditions in sand or soil in the greenhouse. Fewer seedlings were attacked and infection was confined mainly to dark-brown lesions of varying size (Fig. 1, G). These lesions developed chiefly on the roots and hypocotyls, and to a lesser extent on the cotyledons. Severely infected seedlings usually did not emerge. Stunted and malformed seedlings were sometimes produced by weak or damaged seeds, but they lacked the distinct lesioning caused by the pathogen. When the lesioning was not extensive, the seedlings sometimes recovered and grew into fairly strong plants. Symptoms similar to those just described were produced by artificial inoculation of the seed or by infesting the soil with the pathogen, but the damage was usually more severe than that arising from naturally infected seed. In both cases the pathogen was consistently isolated from the lesioned seedlings.

The influence of soil temperature and moisture on infection of alfalfa seedlings by Ascochyta imperfecta was studied in soil-temperature-control tanks. Naturally infested seed was planted in crocks of steam-sterilized soil (3 parts of black loam and one part of sand) held at 4 soil temperatures, each averaging 11°, 17°, 21°, and 26° C. In one series at each temperature the soil was kept dry (approximately 40 per cent m.h.c.), and in the other series relatively moist (60 per cent m.h.c.). At each temperature and moisture, seed treated with Arasan, and untreated, was

TABLE 4.—Relative virulence of isolates of Ascochyta imperfecta on the roots of Medicago, Melilotus, and Trifolium. (Field tests.)

Isolates			Ğ	Generala pathogenicity tests	genicity tests			Special ^b test	
	Number	Me	Medicago	Melilotus	otus	Trif	Trifolium	Medicago	
Source	tested	sativa	falcata	alba	officinalis	pratense	hybridum	sativa	
		Pct.	Pct.	Pct.	Pct.	Pet.	Pct.	Pct.	
Medicago, roots	, ∞	21	16	17	20	19	12	28.0	
	9	23		14				26.4	
	c 1	19		15		1		18.6	
	©1	61		16			-	29.4	
	 1	20	•	17				56.6	
Melilotus, stems	-1	20		50	1	1			
Lathyrus, leaves	I	24		50	1	1		30.8	
Soil	27	21		18	1			33.8	
Average rating		21		17	*****			27.7	

^a Average disease rating on 10 plants of one or more isolates from different sources.

^b Average disease rating of 100 plants of Grimm alfalfa inoculated with one isolate from each source. F value for isolates 4.12, 5 per cent point 2.51, 1 per cent point 3.67. Difference required for significance (twice S.E. of difference) 6.4.

planted (20 seeds per crock), and in a third set the soil was infested at seed level with the pathogen (10 grams of soil inoculum per crock). Emergence was highest with the treated seed, lowest in infested soil, and intermediate when untreated seed was planted (Table 5). In the infested soil, the percentage of lesioned seedlings decreased with rise in temperature. Fewer seedlings emerged and more were diseased in the moist soil than in the dry soil at all temperatures except 26° C. In the steam-sterilized soil, lesioning occurred only on a few seedlings grown from untreated seed at 11° and 17° C. These results indicate that relatively cool, moist soil is most favorable for infection of alfalfa seedlings by A. imperfecta.

Ascochyta imperfecta was the only pathogen isolated from alfalfa seed

TABLE 5.—Effect of soil temperature and soil moisture on pre- and post-emergence attack of alfalfa seedlings by Ascochyta imperfecta. (Naturally infected seed from the 1941 crop treated with Arasan, untreated, and planted in infested soil.)

		Emergence		Seedlings diseased				
Temperature Moisture	Se	ed	Ct . 27	Se	ed	Qo:1		
	Treated	Untreated	Soil infested	Treated	Un- treated	- Soil infested		
°C.	Pct.	Pet.	Pct.	Pct.	Pet.	Pct.		
11 Dry	68	56	47	0	2	7		
Moist	64	50	43	0	3	12		
17 Dry	66	57	41	0	3	6		
Moist	60	54	36	0	3	8		
21 Dry	63	52	52	0	0	4		
Moist	61	48	38	0	0	6		
26 Dry	68	64	50	0	0	3		
Moist	70	63	50	0	0 / 2	3		
Average	65.0	55.5	44.6	0	1.4	6.1		

(Table 1) which proved parasitic to the seedlings. Stagonospora meliloti and Sclerotinia sclerotiorum occurred occasionally in the seed samples, but had no apparent effect on germination or growth. Alternaria spp. and other fungi and bacteria commonly isolated from the seed also appeared to be purely saprophytic.

SEED TREATMENT STUDIES

The effect of various fungicides on alfalfa seed infected with Ascochyta imperfecta was studied in laboratory, greenhouse, and field experiments. Treatments were made by thoroughly shaking the seed with a slight excess of the chemical dust, which was afterwards screened off. The relative efficiency of each treatment in destroying the pathogen was determined by plating out 100 seeds of each sample before and after treatment. In the greenhouse experiments, five 100-seed replicates of each sample and treatment were planted in sand in cardboard boxes. Similar experiments were conducted in the field in replicated 10-foot rows. Notes on emergence and disease development were taken 2 weeks after planting.

In the greenhouse experiments many samples of alfalfa seed were treated with Arasan, New Improved Ceresan (full strength and diluted to 1 per cent ethyl mercury phosphate with talc), Semesan, and Spergon. The results obtained in a representative test with 3 severely-infected samples are given in table 6. All fungicides tested were reasonably effective in inhibiting the pathogen, although it was usually isolated from a few seeds after treatment with Semesan and Spergon. Also, very few of the seedlings grown from treated seed were parasitized. Full strength New Improved Ceresan (5 per cent ethyl mercury phosphate), however, had an injurious effect on germination. Some samples were also slightly injured by Semesan and Spergon. With Arasan and diluted New Improved Ceresan (1 per cent ethyl mercury phosphate) there was no evidence of

TABLE 6.—Effect of different chemical treatments on three samples of alfalfa seed naturally infected with Ascochyta imperfecta. (Greenhouse experiment.)

		Seeds rfected	[a	E	mergen	eeb		eedlir liseas		Chemical
Treatment	Sai	nple	No.	S	ample 1	Vo.	Sa	mple	No.	injury
	1	2	3	1	2	3	1	2	3	
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	
Arasan New Improved	0	0	0	61.4	66.2	60.8	0	0	0	Trace
Ceresan, 1 Pct.	1	0	1	60.2	62.4	65.0	0	0	0	Trace
Ceresan, 5 Pct.	. 0	0	0	50.2	54.4	54.8	0	- 0	0	Moderate
Semesan	3	6	1	55.8	59.2	61.0	1	2	0	Slight
Spergon Control	4	1	2	61.0	60.2	59.8	1	0	1	Slight
(non-treated)	38	38	43	53.2	51.8	58.2	5	4	4	

a Based on isolations from 100 seeds of each sample.

b F value for treatments 12.11, 5 per cent point 2.35, 1 per cent point 3.29. Difference required for significance (twice S.E. of difference) 5.8.

chemical injury, and emergence in all three samples, when treated, was significantly higher than in the nontreated controls. As previously noted (Table 5), Arasan also increased the emergence and prevented blight of the seedlings under varying conditions of soil temperature and soil moisture.

In field experiments with a large number of samples the effects of seed treatment were much more variable than in the greenhouse. Treatment with Arasan, New Improved Ceresan, Semesan, and Spergon increased emergence in some samples but not in others. The beneficial effect was not entirely due to control of the pre-emergence blight caused by Ascochyta imperfecta, since seed treatment stimulated germination in samples containing both high and low percentages of infected seed. The pathogen apparently caused at least part of the pre-emergence blight that occurred in some of the nontreated controls, but it did not produce lesions on the emerged seedlings in the field. Full strength New Improved Ceresan had only a slight detrimental effect on germination in the field, and the other treatments apparently caused no injury.

LONGEVITY OF THE PATHOGEN

The longevity of Ascochyta imperfecta in dry stems and leaves of alfalfa was studied by isolating at yearly intervals from herbarium specimens stored at room temperature. Spore and tissue isolations were made from each sample. The viability of the spores from the 10 samples of stems studied remained high for about 4 years after date of collection, after which it decreased rapidly. In 2 samples a few spores were viable after 6 years, but none germinated at the end of 7 years. Isolations from tissues were made with great difficulty from stems stored 5 years, and none were obtained by the end of the sixth year. The pathogen also remained viable for about 5 years in the tissues of infected alfalfa leaves.

In the case of alfalfa seed, isolations were made at yearly intervals from samples naturally infected with Ascochyta imperfecta. These samples were stored in seed envelopes at room temperature. The 16 samples harvested in 1941 showed an average infection of the seeds of 25 per cent after being stored for one year, 16 per cent after 2 years, and 6 per cent after 3 years. However, at the end of 3 years the fungus was not isolated from samples which originally contained less than 5 per cent of infected seeds. The most severely infected sample contained about 54 per cent of infected seeds during the first year of storage, 40 per cent after 2 years, and 20 per cent after 3 years. The pathogen remained viable for 3 years in 2 out of 4 samples from the 1940 crop, and for 4 years in only one of the samples. In the latter sample the original infected seed content of about 25 per cent decreased to 2 per cent during the 4-year period. In comparison with these results, Crosier (6) found that the number of seeds of hairy vetch infected with Ascochyta pisi decreased continuously during a storage period of 5 years. Sprague (15), however, isolated this pathogen from 9-year-old seed of Vicia faba. Longevity tests on A. imperfecta have apparently not been previously reported, and the present studies are being continued.

TAXONOMY

Considerable confusion exists in the literature concerning the identity of the causal agent of black stem of alfalfa. It was first described as Ascochyta imperfecta by Peck (12) in 1911, and this name was recognized by Sprague (15) in 1929 in his study of the leguminous Ascochytae. Johnson and Valleau (7), in 1933, however, attributed a similar disease of alfalfa in Kentucky to Phoma medicaginis Malbr. & Roum., and the latter name was accepted by Remsberg and Hungerford (14) and other workers. In 1936, Toovey, Waterston, and Brooks (17), in Britain, made a careful study of all available specimens and cultures, including the pathogen from Kentucky, and concluded that they all belonged to the same species and should be properly referred to as Ascochyta imperfecta Peck. These workers suggested that certain other species of Ascochyta, Diplodia, Phoma, and Phyllosticta described on alfalfa in Europe might

also be identical with A. imperfecta. This name has been used in the Canadian plant disease survey reports since 1938 (3), and has now become generally recognized in the literature.

Description of the Alberta Isolates

Cultures of Ascochyta imperfecta isolated from various hosts and sources in Alberta conformed fairly closely to the original species description by Peck (12), and also fell within the range of the characteristics described by Toovey, Waterston, and Brooks (17). These isolates, however, differed markedly from each other in certain cultural characteristics (Fig. 2, C). The colonies ranged from light grey to dark-brownish grey, usually with a characteristic olive tinge. Some isolates grew much more rapidly than others, possibly due to different rates of staling. The colony diameter of the different isolates ranged from 25 to 65 mm. after incubation for 7 days at 22° C. Pycnidia varied in number, size, color, and in position in the culture. They were usually abundant, but certain isolates characteristically produced them very sparingly, even when cultured on sweetclover stems or other media particularly favorable for their fruiting. pycnospores were continuous or one-septate, depending on the isolate and on cultural conditions, and were generally larger than those produced on Chlamydospores were very numerous, and a few large, brownish, one-septate, spore-like bodies sometimes occurred at the surface of the medium. The white crystal aggregates described by other workers (7, 17) were produced in the medium in varying numbers by different isolates.

Variants frequently occurred in the form of sectors or patches in testtube and plate cultures (Fig. 2, C). These variants differed from the parent culture in color, growth rate, and pycnidial production. It is possible that variants of this fungus also occur commonly in nature, which would account for many of the differences observed between isolates.

There was no evidence of any relationship between the cultural characteristics of an isolate and its source. For example, the full range of characteristics described occurred among the isolates from both stems and roots of alfalfa. The composition of the medium as well as various environmental conditions had a marked influence on the cultural behavior of the fungus, but, in general, they did not mask the differences between isolates.

In temperature studies, most of the Alberta isolates of Ascochyta imperfecta grew best at 20°-22° C., which is in close agreement with the results of Peterson and Melchers (13). Pyenidia and spores generally developed most rapidly at about 17° C. At 5° C. growth was relatively slow and sparse, but the pyenidia were eventually as numerous as at higher temperatures. When cultured on sweet-clover stems, most isolates produced numerous pyenidia and caused a blackening of the stems that was particularly evident at 17° C.

The Perfect Stage

Over 50 samples of overwintered stems of alfalfa collected in different sections of Alberta were examined in an effort to find the perfect stage of *Ascochyta imperfecta*. Also, several unsuccessful attempts were made to induce perithecial development by subjecting infected stems and cultures to varying conditions of temperature, moisture, and light.

Perithecia of Leptosphaeria, Mycosphaerella, Pleospora, and other fungi were not infrequently found in association with the pycnidia of Ascochuta imperfecta on the overwintered stems. No attempt was made to identify most of these fungi, since cultural tests indicated that they had no genetic connection with the pathogen. However, a special study was made of a species of *Pleospora* found on 3 of the overwintered samples. This fungus corresponded very closely to the description of P. rehmiana (Staritz) Sacc.. reported by Remsberg and Hungerford (14) as the perfect stage of the black-stem pathogen (Phoma medicaginis Malbr. and Roum.) in Idaho. As mentioned above, this fungus is now considered identical with A. imperfecta. These workers found perithecia of P. rehmiana on old stems of alfalfa, and in sweet-clover-stem cultures of the pathogen held for 3 months at $0^{\circ}-5^{\circ}$ C. They stated that the original single-ascospore isolates were somewhat different from single-spore isolates of the pathogen, but that typical pycnidia and pycnospores developed after culture on sweetclover stems. Single-ascospore isolates from perithecia on Alberta specimens, however, developed into cultures unlike those of A. imperfecta. These isolates also retained their identity and failed to produce pycnidia or pycnospores under a wide range of conditions, which included culture for several months on sweet-clover stems at 0°-25° C. Perithecia, asci, and ascospores, closely resembling those found in nature, eventually developed in sweet-clover-stem cultures held at 0° to 18° C.

The Alberta isolates of the *Pleospora rehmiana*-like fungus also failed to attack the stems and leaves of alfalfa in several inoculation experiments. They caused very slight infection of alfalfa and sweet-clover roots, and the original fungus was reisolated from the lesions.

From this evidence it is concluded that the *Pleospora sp.* resembling *P. rehmiana*, occasionally found on overwintered stems of alfalfa in Alberta, has no connection with *Ascochyta imperfecta*. Johnson and Valleau (7) were also unable to establish a genetic connection between *Pleospora* and the black-stem pathogen in Kentucky.

DISCUSSION

Present evidence indicates that Ascochyta imperfecta is primarily a parasite of the stems and leaves of alfalfa, and is of doubtful importance on other naturally infected hosts. Although able to cause root rot of legumes under experimental conditions, it usually occurs in nature in root lesions produced by other fungi. For this reason it is regarded as of less importance in Alberta than any of the root-rotting pathogens pre-

viously reported (5). However, there are indications that it may increase the root damage started in the early spring by the low-temperature fungi with which it is usually associated. Infection of alfalfa seedlings by A. imperfecta is also of relatively minor importance under average field conditions in Alberta. Pre-emergence attack, however, sometimes results in lowered seed vitality, and this aspect requires further study.

Diseased alfalfa stems, on which numerous pycnidia of Ascochyta imperfecta develop in the late fall, are undoubtedly the most important source of inoculum. Under moist conditions in the spring the spores exude from these pycnidia and are readily disseminated by rain drops to start a new cycle of infection on the young growth. The frequent occurrence of A imperfecta in lesions at or near the crowns suggests that infected crop residue decaying near the surface is also the source of inoculum for alfalfa It is natural that as alfalfa stands become older and plant debris accumulates, the pathogen increases in abundance in the surface soil and may also spread downward to a limited extent. Apparently it is unable to persist very long in the soil after alfalfa has been plowed, which indicates the value of crop rotation. However, it may remain viable for at least 5 years on dried stems and leaves of alfalfa, and for about 3 years on the seed. This suggests that the hay, meal, and seed obtained from infected alfalfa stands may harbor the fungus and aid in its dissemination. Infected seed, in particular, may serve to introduce the pathogen into areas or fields where it is not already present. Hence, it is possible that the distribution of infected seed may be partly responsible for the general prevalence of stem and leaf infection by A. imperfecta in alfalfa stands of all ages.

As suggested by Allison and Torrie (1), the chemical treatment of alfalfa seed requires much further study before it can be recommended as a general practice. The effect of fungicides on seed germination and on the nodule-producing bacteria are factors that must be considered. In Alberta, seedling blight of alfalfa caused by Ascochyta imperfecta or other seed- and soil-borne pathogens does not yet seem of sufficient importance to warrant general seed treatment. Further investigation is required, however, to determine the possible value of testing alfalfa seed for the presence of A. imperfecta, and of treating heavily-infected samples in order to reduce the spread of the pathogen.

SUMMARY

In field, greenhouse, and laboratory studies, Ascochyta imperfecta, the fungus causing black stem of alfalfa, parasitized the roots of alfalfa and other legumes, and also seedlings of alfalfa, and it was both seed- and soil-borne.

Medicago sativa was more susceptible to stem, leaf, and root infection than any other host studied. Melilotus spp. and Trifolium pratense were slightly susceptible, and T. hybridum rather resistant. Occasionally the

pathogen was isolated from stems of Vicia americana and leaves of Lathurus

Cool, moist conditions were most favorable for disease development on stems, leaves, and seedlings of alfalfa. The pathogen did not attack the roots of dormant plants at soil temperatures near freezing.

The degrees of seed and stem infection were directly correlated. Ascochuta imperfecta was isolated from as much as 40 per cent of the seeds of 49 out of 97 random samples of alfalfa seed. New Improved Ceresan, diluted to one per cent ethyl mercury phosphate, and Arasan were more effective than other seed treatments tested.

The fungus was prevalent in the surface soil of alfalfa fields, but disappeared 2 years after the sod was plowed. Moreover, it was not isolated from the soil of cereal rotations, virgin prairie, or virgin woods. It persisted on dry stems and leaves of alfalfa for at least 5 years, and on alfalfa seed for about 3 years.

There was no evidence of host specificity, since isolates of Ascochyta imperfecta, obtained from various hosts, different parts of the same host, and from soil, exhibited similar differences in virulence and cultural characteristics.

Various Ascomycetes, including a Pleospora closely resembling P. rehmiana, were found associated with the pyenidia of A. imperfecta on overwintered stems of alfalfa, but, in no case was proof of a genetic connection established.

The writer is indebted to Dr. G. B. Sanford, Pathologist-in-Charge, Dominion Laboratory of Plant Pathology, Edmonton, Alberta, for helpful advice throughout this investigation.

DOMINION LABORATORY OF PLANT PATHOLOGY. EDMONTON, ALBERTA, CANADA.

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AMERICAN PHYTOPATHOLOGICAL SOCIETY

1945 MEETING CANCELED

The Office of Defense Transportation has announced that travel restrictions will not be lifted for several months after official declaration of the end of the war. For this reason the Council voted that it was inadvisable to hold the 1945 meeting, scheduled for December 10–12, in Cincinnati, Ohio.

WILDFIRE DISEASE OF SOYBEANS¹

WILLIAM B. ALLINGTON²

(Accepted for publication June 10, 1945)

INTRODUCTION AND REVIEW OF LITERATURE

An intensive soybean disease survey was made by the United States Regional Soybean Laboratory in the summer of 1943, covering many of the heavy producing areas. Of particular interest was a disease identified as wildfire, caused by *Pseudomonas tabaci* (Wolf and Foster) Stapp.

Three references in the literature report soybeans (Soja max Piper) susceptible to this parasite when artificially inoculated (1, 8, 10). In one instance attempts to re-isolate the organism and establish definite proof of pathogenicity were not reported (8). Valleau et al. (10) have reported ten successful passages of the organism in soybeans growing in the greenhouse. To the writer's knowledge, however, the disease has not been previously recognized to cause extensive damage to soybeans in the field. The total damage observed in 1943 and 1944 was not significant. However, in individual fields, damage was severe enough to make evident the potentialities of this disease on soybeans and to emphasize the need for careful study.

SYMPTOMS

Symptoms are so characteristic that this criterion alone is usually sufficient for identification of wildfire. The necrotic spots on the leaves are variable in size and are nearly always surrounded by a striking wide yellow halo (Fig. 1). At times the halo, as on tobacco, may be rather indistinct. This condition is thought to be the result of rapid invasion by the bacteria to cause a breakdown of the tissue before the chlorophyll-destroying exotoxin has had time to destroy the chlorophyll. In such instances the necrotic spot is dark brown to black as contrasted to the usual light brown. The lesions, in suitable damp weather, tend to enlarge and coalesce, and eventually they involve large areas of the leaf. Defoliation, starting on the lower leaves, is common and under certain conditions may progress until few leaves remain. So far as is known the infection on soybean is confined to the leaves.

The infection in most fields is usually limited to definite areas, which may be a few feet in diameter or several acres. Few fields have been observed to be uniformly infected throughout. This is thought to be partly due to variation in environmental factors such as soil fertility and drainage.

² Associate Pathologist, U. S. Regional Soybean Laboratory, Urbana, Illinois.

¹ A publication by the U. S. Regional Soybean Laboratory, a cooperative organization participated in by the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration; and the Agricultural Experiment Stations of Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee, Texas, Virginia, and Wisconsin.

OCCURRENCE AND DISTRIBUTION

Wildfire was observed in all the major soybean-producing areas, except possibly North Carolina, in 1943 or 1944. The first specimen to come to the writer's attention was collected in northern Mississippi in July, 1943. In early August, 1943, severe infections were found in Arkansas, Tennessee, Alabama, and Georgia. In late August and early September of the same year, infection was observed in Iowa, Illinois, Indiana, and Ohio. In 1944, the disease was reported on soybeans from most of the North and South

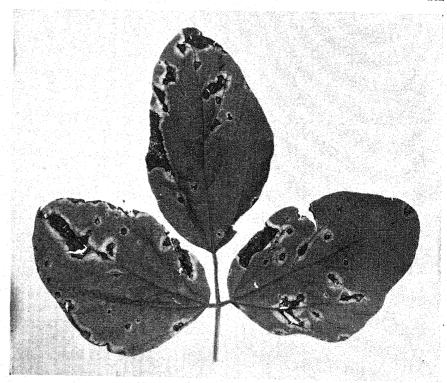


Fig. 1. Typical wildfire on soybean from natural infection in the field. The striking yellow halo around the necrotic spots is the chief distinguishing feature. Note coalescence of necrotic areas.

Central States. Extensive infection in 1943 and 1944 was rare before August, but it is not known whether wildfire is typically a late season disease on soybeans.

The most extensive infection observed to date over a wide area was in central Iowa in August, 1944. Wildfire is not known definitely to have occurred on soybeans before 1943, but it probably has been present in isolated areas for several years. Definite increase in the disease on soybeans over the country has been observed in the last two years. Should this continue, it may well be a menace to the soybean crop.

THE CAUSAL ORGANISM

Extensive tests have been made with the soybean wildfire organism to compare it with the tobacco wildfire bacterium. Studies of their morphological, physiological, serological, and pathological characteristics are here reported.

Morphology and Physiology.—Six isolates of the soybean wildfire organism were compared in pure culture with two isolates of Pseudomonas tabaci (Wolf and Foster) Stapp obtained from other laboratories. The cultural differences within each group were as great as the differences between the

groups.

The soybean wildfire organism is a white, Gram-negative rod, $0.86 \times 3.0~\mu$, motile by 1–5 polar and bipolar flagella. Spores are absent. The colony type is indistinguishable from *Pseudomonas tabaci* on potato-dextrose agar at pH 7.0. Spontaneous dissociation into rough and smooth colonies was frequent on the various culture media.

The soybean wildfire organism liquefied gelatin readily. Litmus milk was turned blue in 4 days, the litmus being eventually reduced with coagulation. Hydrogen sulphide was not produced in peptone broth. Indole was not produced in broth containing tryptophane (Gore method). Starch was not hydrolyzed. Green fluorescence was commonly observed on a variety of liquid media.

No definite differences were found between the soybean isolates and the tobacco isolates in their ability to utilize the following carbon and nitrogen sources: lactose, levulose, inulin, mannite, maltose, glycerol, l-arabinose, l-xylose, d-galactose, dextrose, sucrose, ammonium sulphate, potassium nitrate, potassium nitrite, l-cystine, glycine, succinimide, acetamide, and urea. The results were in substantial agreement with those reported by Braun (2). Slight variation was observed in the ability of the isolates to utilize these compounds. However, the difference between the soybean isolates and the tobacco isolates was not greater than the differences within each group.

Serology.—The agglutination and agglutinin absorption tests were used to further test the relationship of the soybean and tobacco wildfire organisms. The agglutinin absorption test is considered by many to be the most accurate test for the identity of organisms. However, it may be preferable to use these tests in conjunction with the others reported, and to draw conclusions in regard to relationships from the general results produced. Bacteria are known to undergo changes, in pure culture at least, which can influence the investigator to such an extent that he may be led to create new species or varieties or eliminate species and varieties unwisely, unless he has sufficient information of various types upon which to base his judgment.

The serological relationships of four soybean isolates and two tobacco isolates were compared. Isolates S1, S2, and S6 originated from diseased soybeans from central Illinois, while S7 was isolated from diseased soybeans from Alabama. Tobacco isolates T10 and T11 were obtained from the Divi-

sion of Tobacco Investigations, U. S. Department of Agriculture. Cultures were from single colonies but were not single-celled. Their pathogenicity was established and checked periodically during the experiment and they were always highly pathogenic to both soybeans and tobacco. Only the "smooth" forms of the cultures were used for injections.

Rabbits were used for the production of the immune sera. Before the first injections the normal sera from these animals were tested and found to be void of normal agglutinins for the organisms studied. The bacterial cultures used for the immunization of the rabbits and the serological tests were grown for 24 hours on potato-glucose agar. Suspensions of the bacteria for injection into the animals were made in 0.85 per cent NaCl solution and filtered through sterile filter paper. The turbidity was standardized to conceal a 2-mm. loop made of No. 26 B. & S. gauge wire at a depth of 12 mm.

TABLE 1.—Results of agglutination tests with sera from rabbits injected with 4 isolates of soybean wildfire bacteria and two isolates of tobacco wildfire bacteria, Pseudomonas tabaci (Wolf & Foster) Stapp

Maximum dilution at which agglutination occurred								
Antiseraa				Isol	ate			
		S1	S2	86	S7	T10	T11	
S1		1280	1280	2560	2560	2560	1280	
S2		1280	1280	2560	2560	1280	1280	
S6		2560	1280	1280	2560	640	640	
87		2560	1280	1280	2560	640	1280	
T10		1280	1280	1280	1280	1280	1280	
T11		2560	2560	2560	2560	2560	1280	
Normal seru	m	0	0	0	0	0	0	

^a Antisera S1, S2, S6, S7 obtained from rabbits inoculated with soybean wildfire isolates. Antisera T10 and T11 from rabbits inoculated with two tobacco wildfire isolates. Homologous antisera and isolates bear the same numbers.

The rabbits received 7 intravenous injections, at intervals of 4 to 5 days. The amount of each injection was, respectively: 0.5 cc., 1 cc., 1.5 cc., 2.0 cc., 2.5 cc., 3.0 cc., and 4.0 cc. Ten days after the last injection, the rabbits were bled from the ear vein.

The results of the macroscopic agglutination tests are in table 1. Although the titre of the sera are slightly low, the data indicate the close relationship between the isolates from soybean and those from tobacco, and one cannot differentiate them by this test.

The agglutinin absorption test was made with the same antisera as the agglutination tests. The minimum absorbing dose of antigen was determined for each serum with its homologous organism. Bacteria were suspended in a phenolized saline solution (0.5 per cent phenol) which was adjusted to different densities varying from 3.0 to 8.0 on the McFarland nephelometer scale. One part of nondiluted antiserum was added to 39 parts of each of the suspensions, giving a serum dilution of 1-40 in each

case. The tubes were incubated at 37° C. for 2 hours, centrifuged, and the clear supernatant liquid removed to clean, sterile, centrifuge tubes. Bacteria from the packed centrifuge sediment of a broth culture were added to produce again the original turbidity in each tube. The tubes were again incubated at 37° C. for 2 hours, centrifuged, the clear supernatant liquid removed and reabsorbed with bacteria. After a subsequent 2-hr. incubation at 37° C. they were placed in the refrigerator over night. The following morning, they were centrifuged and the supernatant was used for agglutination tests with the homologous organisms, with antiserum dilutions through

TABLE 2.—Results of agglutinin absorption tests with cultures of two soybean wildfire isolates and cultures of two tobacco wildfire isolates

		Maximum dilution at which agglutination occurred							
Antiseraa	Absorbing antigen		Isolate						
		86	S 7	T10	T11				
S6	S6 S7		********	.,,,,,,					
	T10 T11		•••••						
S7	S6 S7		- 10		*				
	T10 T11								
T10	S6			**************************************	*******				
	S7 T10		160						
	T11								
T11	\$6 \$7		320	320 80	16 0				
	T10 T11			********					

^a Antisera S6 and S7 obtained from rabbits inoculated with soybean wildfire isolates. Sera T10 and T11 from rabbits inoculated with two tobacco wildfire isolates. Homologous antisera and isolates bear the same numbers.

640. The lowest turbidity to have completely absorbed the agglutinins was considered the minimum absorbing dose for that serum. By using suspensions about four times as heavy as the minimum absorbing dose, portions of each antiserum were absorbed with one of the six cultures. Each absorbed antiserum then was tested for agglutinins for all six isolates. The results of the tests are in table 2.

In most cases reciprocal agglutinin absorption occurred. It is believed that the five positive tests in table 2 are due to inherent variations in the method and are of no consequence, especially since there is no consistency. If these results do indicate differences in the organisms studied, one can safely say that the difference between the two tobacco isolates is as great as between the soybean and tobacco isolates.

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Pathogenicity.—In view of the recent work on the relationship of water-soaking of tissue to infection by bacteria, caution should be exercised in the interpretation of data in regard to pathogenicity. This subject will be discussed later in the paper. The soybean wildfire isolates were introduced into watersoaked leaf tissue of tobacco or soybean in order to obtain infection. Practically no infection results with these or with the tobacco isolates without some degree of watersoaking. Figure 2 shows infection on tobacco with the soybean and tobacco isolates. After the development of typical wildfire symptoms on soybean and tobacco plants, portions of the infected tissue were macerated, diluted, and plated out on potato-dextrose agar at pH 7.0. The organism was readily reisolated. Subsequent passages and the number of bacteria obtained from the lesions left no doubt as to the causal agent.

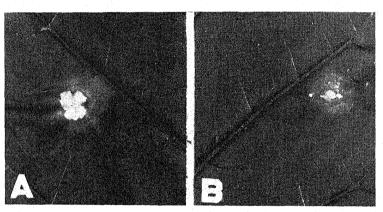


Fig. 2. A. Wildfire lesion on tobacco caused by an isolate from soybean. B. Wildfire lesion on tobacco caused by an isolate from tobacco. Tobacco was artificially inoculated by atomizing. Fundamentally, no differences existed between the isolates.

The virulence of the wildfire isolates on tobacco has been reported to vary greatly, and the soybean isolates were no exception in this respect. The ability of this parasite to kill tissue and to produce the powerful chlorophyll destroying exotoxin so characteristic of the organism varied extensively with the different isolates.

The exotoxin is produced abundantly in pure culture, and by heating as well as by filtration the organism was separated from it. The exotoxin was tested several times on living plants in the greenhouse and found to be active when introduced into leaves by the needle prick method.

Extensive inoculation tests in the greenhouse and field for two years have failed to disclose any difference in symptoms produced by the soybean and tobacco wildfire isolates.

THE INFECTION PROCESS

A new and not too generally recognized aspect of infection by leaf-spot bacteria, i.e., the role of watersoaked tissues, has been studied and reported on by a relatively few investigators (3, 6, 10). The writer has found water-soaking of great consequence in the penetration of soybean leaves by *Pseudo-monas tabaci*.

Soybean leaves become watersoaked in the field either by a beating rain or by internal physiological phenomena. Often this watersoaking is of limited extent and of short duration, and can easily pass unnoticed. Diachun et al. (5) have described the manner in which liquids, containing chemicals, ink, etc., penetrate watersoaked tobacco leaves. Approximately the same experiment was performed with soybeans. Three methods of artificially watersoaking soybean leaves were employed in this study with the aim of simulating physiologic and storm watersoaking. Obviously natural conditions cannot be duplicated exactly but it is hoped that the methods, materials, and results reported here will suffice to demonstrate the writer's views on the infection phenomena as they occur in nature.

Two methods have been employed to watersoak leaf tissue experimentally without beating the water against the epidermis as occurs in heavy rains. One is the hypodermic needle method (Fig. 4, C). Water can readily be injected into the intercellular spaces by inserting the needle near a vein on the lower leaf surface. The other method is the insertion of the cut stem of the plant into a water-tight connection and its subjection to high water pressure as described by Johnson (6). Both methods produce a physiologic watersoaking similar to the type occurring in the field in the absence of beating rain.

Watersoaked tissue will absorb a liquid from the surface of the leaf very readily when a junction is established between the intercellular and the external liquid (Fig. 3). Penetration and dispersion within the watersoaked area of the leaf is very rapid, usually requiring only a few The forces operating to pull the liquid into the leaf are unknown but appear to be related to capillarity. Liquid junctions, resulting in bacterial penetration apparently are not so common in this type of watersoaking as in the storm type. Without mechanical injury, drops of bacterial suspension or other liquids may stand indefinitely on a physiologically watersoaked area without penetration occurring (Fig. 4, A and B). If the water by the application of internal pressure has been forced out through the stomata or old wounds in the leaf then penetration is immediate. This rarely is observed in nature. On healthy soybeans in the field, physiological watersoaking is usually confined to the base of the leaflet at the point of attachment. It may be observed early in the morning, during the growing season, especially if heavy dew is present. However, in severe epidemics of wildfire, only a few of the leaves may have characteristic infections at the base where this watersoaking has occurred. It is doubtful, therefore, if this type of watersoaking is of much consequence as far as initial infection of soybeans is concerned. Johnson (7), working with tobacco, has concluded that this type of watersoaking is very important in initial infection, and that prolonged periods of watersoaking are essential for the production of large lesions.

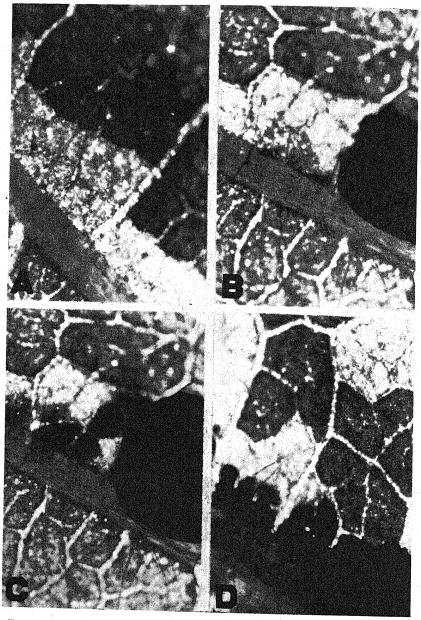


Fig. 3. Photomicrographs showing the progress of India ink in soybean leaf tissue artificially watersoaked by the hypodermic needle method (Fig. 4, C). Photographed with transmitted light, so that the translucent watersoaked tissue appears light as conink has been placed over an unwounded portion of the watersoaked area. C. After wounding, the ink penetrates the leaf tissue very rapidly. D. Further penetration of ink. The process of penetration, as illustrated in C and D, may take place in less than 10 seconds.

Beating rains are observed to be closely connected with wildfire epidemies on soybeans. Under these circumstances, the liquid junction is automatically made between intercellular and surface water when the water is driven into the leaf. Figure 4, A and B, demonstrates how the two types of watersoaking respond when India ink is placed over the watersoaked area when care is taken not to puncture the epidermis with sharp instruments. When watersoaking occurs, there may or may not be mechanical injury to the tissues. Observations have indicated that the condition of the plant is an extremely important factor in regard to the amount of infection. For

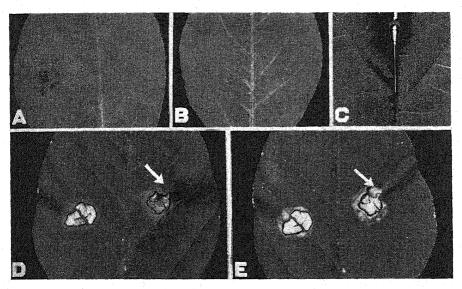


FIG. 4. Soybean leaves illustrating various relationships of watersoaking to infection and development of wildfire. A. A small area was watersoaked by atomizing water against the lower surface. India ink was then placed over the watersoaked tissue for five minutes as well as over the non-watersoaked tissue on the opposite side of the leaf, then gently washed off. Penetration of ink can only be observed where watersoaking existed. B. Leaf previously watersoaked by water pressure from water line through cut stem. India ink was placed over the watersoaked areas on this leaf, as in A, for five minutes. No penetration can be observed. C. Method of watersoaking leaf tissues by use of the hypodermic needle. D and E. Wildfire lesions on the same leaf. E was photographed five days later than D. Inoculation was made by atomizing the bacterial suspension against the lower surface where the infected areas developed. One week after inoculation, the area indicated by the arrow in D was watersoaked as illustrated, by atomizing sterile water. Five days later, as shown in E, the lesion occupied part of this previously watersoaked area (arrow in E). Watersoaking persisted thirty minutes. Note similarity of the lesion on the left in D and E.

example, the condition of the stomata is important as shown by Diachun (4) with tobacco. One year's field trials indicate that soybeans sprayed with bacterial suspensions with a power sprayer are apt to have greater infection if sprayed during the bright sunshine than if sprayed just before daylight or at dusk in the evening. In this connection, it is interesting that at certain times, for example at night, when the stomata on soybeans are closed, storm watersoaking is difficult to produce artificially, yet it is easy in the

daytime. It would appear therefore that the water is driven in through the stomata, and it is only logical to conclude that bacteria penetrate mainly by the same route. On the other hand, physiologic watersoaking occurs on soybeans primarily at night or on extremely dark days, and extensive penetretion through stomats in this

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tration through stomata in this case would be difficult to explain.

The spread of bacteria through leaf tissue is greatly enhanced by watersoaking, as one might conclude from the behavior of ink in watersoaked tissue (Fig. 4, A). This is evident as shown in figure 4, D and E, which illustrates the result of an experiment repeated many times. A leaf was inoculated with a very dilute suspension of wildfire bacteria from a culture 24 hours old. The suspension under considerable pressure was atomized over a small area, thereby watersoaking the tissue in that area and assuring good penetration. One week after inoculation typical wildfire lesions appeared on the leaf, as in figure 4, D. At this time normal tissue adjacent to one of the lesions was watersoaked by atomizing sterile water against the leaf at high pressure. This was done in sunlight and the plant was never removed from the greenhouse bench. Watersoaking persisted for less than 30 minutes. The leaf was rephotographed after 5 days and appeared as in The bacteria had penetrated the watersoaked tissue in a very figure 4, E. short time and the result was an increase in the size of the necrotic spot with a definite zonation at the margin of the old lesion. The lesion that had not received a second watersoaking remained substantially the same, except that slight intensification and growth of the halo was evident after 5 days. Physiologic watersoaking has been observed to operate in a manner very similar to this. Likewise, water from dew absorbed at the margin of static lesions, may cause a very narrow ring of watersoaking and enhance the subsequent spread of bacteria within the normal tissue; the result is reactivation and growth within the lesion, and at times concentric rings are formed.

Penetration and dispersion of the bacteria in watersoaked leaf tissue is surprisingly rapid. Judging from the experimental results obtained, prolonged watersoaking is not essential for the establishment of infection after penetration.

An attempt was made to check directly upon the number of bacteria present in leaf tissue held under various conditions. Soybean leaves were completely watersoaked by directing an atomizer, with sterile water, on the lower surface of the leaves. Leaves of comparable age and position on the plant were used as non-watersoaked checks. A very dilute suspension of Pseudomonas tabaci from a 24-hour potato-dextrose-agar culture was made in a sterile 500-cc. beaker. The watersoaked and non-watersoaked leaves were immersed together in the suspension for 1 minute after which they were exposed to the air for 3 minutes. They were then washed 8–10 times in large beakers of sterile distilled water. Half of each watersoaked leaf was then placed between moist blotters in a moist chamber, the other half was dried in the air for 20–25 minutes, until the watersoaking disappeared. At this time, the non-watersoaked leaf and the half leaf which had

recovered from watersoaking were placed beside the continuously watersoaked leaf between the blotters in a moist chamber. The leaves were subjected to identical conditions except for the watersoaking. After one hour, 10 discs cut at random from each leaf with a sterile cork borer of 6 mm. diameter were completely macerated in 10 cc. sterile water, and adequate dilution was made to facilitate counting of colonies on plates containing 1 cc. of suspension.

The results as shown in table 3 illustrate several facts concerning the role of watersoaking. Obviously penetration was greatly dependent upon watersoaking. The rate of multiplication of the bacteria was greatest within the continuously watersoaked tissue. However, the tissue which was

TABLE 3.—The effect of watersoaking on penetration and the subsequent growth by wildfire bacteria

TI NT-	per disc in from n	mber bacteria 10 leaf discs on-water- d leaves		number bacteria j leaf discs from wa soaked leaves	
Exp. No.	1.01	A (2)	A 01	After 24	hours
	After 1 hr.	After 24 hrs.	After 1 hr.	Watersoaked 1 hr.	Watersoaked 24 hrs.
1 2 3 4 5 6 7 Ave.	3.2 1.0 0.0 50.0 5.1 2.5 0.0 8.8	0.0 20.5 0.2 58.0 23.3 0.9 0.0	19.6 120.6 12.0 208.0 579.0 16.5 27.8 140.5	29700.0 5781.0 200.0 42476.0 180.0 120.0 2045.0 11643.1	13900.0 31700.0 950.0 56175.0 2488.0 5750.0 4844.0 16543.8

watersoaked only during the time of inoculation also supported rapid multiplication. There is so much variability in the results that it is questionable whether this difference with duration of watersoaking is significant. During the time the leaves were recovering from watersoaking, occasionally certain leaves lost more water than others, as judged by appearance. This may be a factor in the variability encountered. It appears that watersoaked or previously watersoaked tissue might be a more favorable place for bacterial growth than tissue that never has been watersoaked. The general averages would indicate approximately one generation in 24 hours in the tissue which never had been watersoaked, as contrasted to between 7 and 8 generations in the tissue previously watersoaked. Considerably more study is necessary to definitely establish this fact.

DISCUSSION

The common occurrence of wildfire on soybeans in the last two years raises many questions in regard to its origin and dissemination. Its occurrence on soybeans never has been reported to be connected with tobacco culture. Three fundamentally different explanations have been advanced to

account for the source of wildfire inoculum for tobacco each year. These explanations are that the inoculum comes primarily from the following: first, the previous year's infected refuse or seed; second, from the soil where the organism has been found to grow epiphytically upon many crop plant roots in addition to lying dormant in the infected refuse or seed (10); and third, from the soil where the organism exists primarily as the common soil saprophyte Pseudomonas fluorescens, mutating or changing suddenly in some manner to become parasitic and to possess all the properties of Ps. tabaci (9). With regard to soybeans the second explanation seems to be the most plausible with the exception of seed transmission. No infection on soybean seed or pods has been observed and no instance of suspected seed transmission has, as yet, been reported.

The incidence of wildfire in isolated spots in large soybean fields, hundreds of miles away from any tobacco growing areas, immediately implies some origin other than previous crops of tobacco. Perhaps the explanation advanced by Valleau et al. (10) for tobacco wildfire, that is, epiphytic growth on various plant roots, could apply to the disease on soybeans. The hypothesis concerning the possible relation to Pseudomonas fluorescens would explain its occurrence; however, it is considered unlikely that the soil saprophyte can readily change to a parasite as virulent as Ps. tabaci.

Until further research has been done no control measures can be recommended. The search for resistant varieties has progressed only one year and resistance has not yet been found.

The serological and pathological results obtained indicate that variability exists in the wildfire organism. However, since this is observed within the group of isolates from soybean and within the group from tobacco, it is reasonable to conclude that the two groups are not sufficiently different for specific or varietal differentiation.

The discovery of the invasion of watersoaked tissue by pathogenic bacteria (3) explained many phenomena in regard to bacterial leaf spots heretofore unaccounted for. A common misconception, however, has been that the tissues concerned must remain watersoaked for several hours. This is not the commonly observed fact in nature and has perhaps delayed the general acceptance of the importance of watersoaked tissue. The importance of physiologic watersoaking on large soybean plants in the field is questionable in so far as it concerns initial infection. On the other hand, the beating of rain on the leaves when the stomata are open, sometimes causes extensive watersoaking which may be followed by severe epidemics of wildfire.

SUMMARY

Wildfire, caused by *Pseudomonas tabaci*, is common on soybeans in most of the commercial soybean growing areas of the United States. The damage in isolated areas is severe. Morphologically, physiologically, serologically, and pathologically the organism isolated from soybeans does not differ from

isolates of *Ps. tabaci* from tobacco, and the two diseases are considered to be caused by the same organism. Watersoaking of soybean leaf tissue greatly enhances penetration of the leaves by *Ps. tabaci* and spread of the bacteria through the tissue. Physiologic watersoaking was not so effective as storm watersoaking in bringing about penetration. Prolonged watersoaking was not necessary for the growth or dispersion of bacteria within the tissues.

U. S. REGIONAL SOYBEAN LABORATORY,

URBANA, ILLINOIS.

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LEAFHOPPER OVIPOSITION, THE CAUSE OF ONE FORM OF APPLE MEASLES¹

JOHN C. DUNEGAN AND DWIGHT ISELY2

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INTRODUCTION

In 1912 Hewitt and Truax (5) reported the occurrence in northwest Arkansas of an apple bark disease which they named "measles." This disease had two distinct forms, the pustular type and the scurfy type. As no pathogenic fungus or bacterium was isolated from the affected tissues they considered the disease a physiological trouble.

In the pustular type, which they found to be the more prevalent form, the disease first appeared as ". . . scattered red pycnidia-like pustules on the young smooth bark. . . ." As the disease progressed the pustules became very numerous and the bark appeared very rough.

A histological study of these pustules revealed that "... they seemed to burrow into the sound tissue underlying them ..." to varying depths depending upon the age of the pustule. The entire pustule appeared to be "... a mass of cork cells with the whole cell nearly filled with some substance which is yellowish brown ..." while "... in the older pustules the tissue in the middle is disorganized leaving a hollow core. ..."

In contrast to these pronounced histological changes associated with the pustular type, Hewitt and Truax found that in the scurfy type "... the phelloderm is irregularly thickened for a considerable area on the branch..." but "... the growth in the scurfy area is at no point as deep as in the pustules, the thickening of the phelloderm amounting to no more than six or eight cells in depth..." Occasionally the two types were intermixed and then the affected area in the scurfy type was "... 5–20 cells deep with the deeper portions in specimens which contain pustules along with the scurf..."

These excerpts from the original description show that the symptoms of the pustular type differ markedly from those of the scurfy type. Furthermore, the pustular type of measles, as described by Hewitt and Truax, is not identical with other obscure apple bark diseases such as the rough-bark or scurfy canker described by Rose (9), the isolated pustular, the aggregate pustular, and canker types described by Rhoads (7), target canker described by Roberts (8), or black pox and internal bark necrosis described by Berg (2). Likewise, the pustular type symptoms are not identical with the symptoms on apple bark resulting from boron deficiency observed by Young and

¹ Research Paper No. 799, Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

² Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and Entomologist, Arkansas Agricultural Experiment Station, respectively.

Winter (11), Burrell (3), and Hildebrand (6), nor with those resulting from the effect of high soluble-salt concentration in the soil, described by Crawford (4) in New Mexico. This becomes especially apparent when it is realized that although Hewitt and Truax emphasized, both in their text and illustrations, the "burrowing tendency" and "hollow core" of the pustules, these striking histological characteristics are not stressed by any of these subsequent investigators. Nevertheless, specimens having all the morphological characteristics ascribed by Hewitt and Truax to the pustular type of apple measles can be found in the apple orchards of northwest Arkansas at the present time. A comparative study of this material is presented.

THE PUSTULAR TYPE OF APPLE MEASLES

The most logical procedure for such a comparative study would be one based on a comparison of present-day specimens with the original material collected by Hewitt and Truax. Unfortunately, none of the original specimens were found in the herbarium of the Department of Plant Pathology, University of Arkansas.³ In lieu of the original specimens the writers based their study on the original description of the pustular type, as well as on unpublished photographs located during the search for the original specimens.

The photographs (Fig. 1, A to E from the files of the Department of Plant Pathology or reproduced from the original paper) illustrate the macroscopic and microscopic appearance of the pustular form of apple measles. Hewitt and Truax were able to strip off the burrowing pustules leaving holes (Fig. 1, D) in the cortex and the intact pustules (Fig. 1, E) attached to the phelloderm and epidermis.

The pustules found at the present time in apple twigs in northwest Arkansas orchards are hemispherical, from 0.25 mm. to 0.75 mm. in diameter, and are raised from 0.1 mm. to 0.25 mm. above the surface of the twig. Superficially they resemble fungus pycnidia, as Hewitt and Truax mentioned in their original account of the disease. In addition to these hemispherical pustules, elongate blister-like swellings are also present on the twigs. These elongate swellings were not mentioned by Hewitt and Truax, yet one of their photographs (reproduced as Fig. 1, A) shows the presence of elongate swellings on the twigs they studied.

In the present study both the pustules and the blister-like swellings began to appear on the current season's growth of a few twigs about the middle of August. In September and October pustules were present on practically all the new growth. They were more numerous on some twigs than on others, but their numbers did not increase after the first frost.

These pustules persist on the twigs for several years. The older pustules are larger, have a less regular outline, and have a slight depression at their center. These changes can be attributed to the subsequent growth of the twig, and it is this growth that makes the surface of the twigs very uneven

³ Hewitt also advised the writers in 1938 that he had none of the original material.

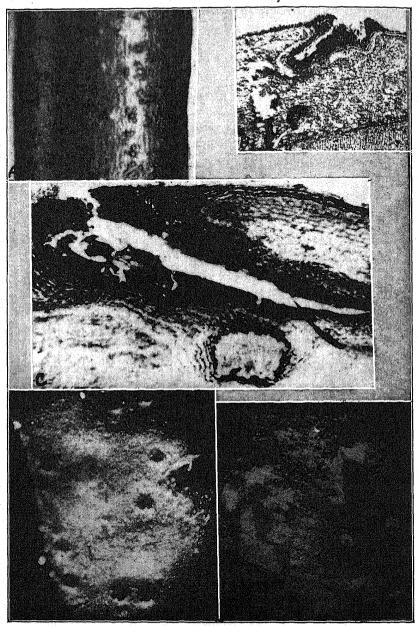


Fig. 1. A. Pustular type of apple measles. Print made from Hewitt's original negative in the files of the Dept. of Plant Pathology, University of Arkansas. B. "Section of an old pustule showing deep burrowing." Legend and figure reproduced from Fig. 8 of Bulletin 112, Arkansas Agricultural Experiment Station. C. "An older pustule showing diseased tissue surrounding a strand of bast." Legend and figure reproduced from Fig. 9 of Bulletin 112, Arkansas Agricultural Experiment Station. D. "Surface view of cortex showing the holes left by removing pustules." Legend and figure reproduced from Fig. 13 of Bulletin 112, Arkansas Agricultural Experiment Station. E. "Surface view of the underside of the phelloderm. The projections are the pustules extending inwards." Legend and figure reproduced from Fig. 12 of Bulletin 112, Arkansas Agricultural Experiment Station. All illustrations and legends in Fig. 1 are reproduced by permission of the Director of the Arkansas Agricultural Experiment Station.

as they grow older. Eventually the pustules lose their distinct outlines and are finally sloughed off, leaving areas of rough bark on these older branches.

CAUSE OF THE PUSTULAR TYPE

The first clue concerning the agent responsible for the pustular type of measles was obtained in 1936, when in examining small pustules on apple twigs received from Oregon it was found that a viscid liquid oozed from a number of the pustules as they were dissected under a binocular microscope. Further dissection revealed the presence in the center of each pustule of an insect egg, which was identified as that of a leafhopper. The twigs were placed in covered battery jars and in due course nymphs of a leafhopper, identified as *Typhlocyba pomaria* McAtee, the white apple leafhopper, emerged from the spots on the twigs.⁴

The elongate blister-like swellings which occur intermixed with the typical isolated pustules of apple measles in Arkansas are readily recognized as having been caused by the deposition of the winter eggs of the white apple leafhopper. Furthermore, the period of formation of the measles pustules coincides with the period of deposition of these eggs and suddenly ceases following the first frost. This suggested that leafhoppers might be involved also in the production of the pustules. The difference in appearance between the elongated blister-like swellings and pustules might be due solely to the angle at which the ovipositor was inserted in the twig.

Washburn (10) reported in 1908 that Ainsle and Webster, who were working with him in Minnesota, had found the winter eggs of a leafhopper in apple twigs during the early part of the 1907 growing season. He described and figured not only what is now regarded as the typical elongate swelling caused by leafhopper oviposition but also an almost "cylindrical pouch" similar to the measles pustules observed by the writers in Arkansas.

When pustules from Arkansas orchards were examined under a binocular microscope, a leafhopper egg was found embedded in each pustule. Histological changes in sections of these pustules were similar to those described and photographed by Hewitt and Truax, as a comparison between Fig. 1, B and C, and Fig. 2, A and B, shows.

These pustules, caused by the insertion of a leafhopper egg, had the "burrowing tendency" observed by Hewitt and Truax. The egg was in the central portion or core of the pustule in many of the sections.

Sections of these pustules on one-year-old and two-year-old wood showed even more clearly the "burrowing tendency" and the "hollow core," as well as the tendency for the spot to involve the pericyclic fibers (Fig. 2, C).

In 3-year-old and 4-year-old wood the subsequent growth of the twigs had produced further marked changes in the pustules themselves. The hollow core disappeared (Fig. 2, D) as the pressure from the twig growth forced the walls of the cavity together, and finally, in the oldest pustules examined, only a solid mass of discolored cells marked the site of the original egg cavity.

⁴ The junior writer (1) had been engaged in a study of the biology of leafhoppers in the Ozarks for several years previously and had reared a number of series of Typhlocyba pomaria in all its stages.

It was difficult to detect fragments of the egg shell in cross sections of this mass of discolored cells, but by removing layer after layer of the sur-

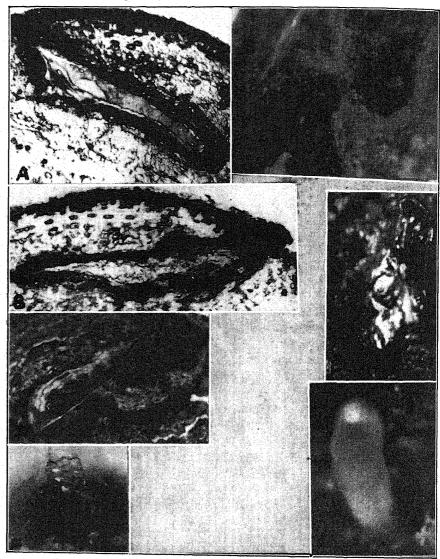


Fig. 2. A and B. Cross sections of pustules showing eggs of Typhlocyba pomaria embedded in tissues of Delicious apple twigs. Note the "burrowing tendency." C. Section showing pericyclic fibers adjacent to a pustule enveloped by a wound periderm. D. Section of older pustule showing the egg cavity being obliterated. E. Outer tissues of old pustule dissected away, revealing fragments of leafhopper egg in the central core of the pustule. F. Fragment of egg shell projecting from central core after surrounding tissues had been dissected away. G. Leafhopper egg attached to underside of epidermis after being withdrawn from young pustule.

rounding tissues under a binocular microscope it was possible to find portions of the egg shell (Fig. 2, E and F) in the center of the pustules.

In contrast to the difficulties encountered with the older pustules, it was relatively easy to withdraw the egg intact from the young pustules (Fig. 2, G), leaving visible a hole in the cortical tissues.

Hewitt and Truax were able to withdraw the entire pustule by soaking the twigs in an aqueous solution of sulphurous acid. Their illustration (reproduced as Fig. 1, E) shows dark spots in the center of a number of the pustules. The writers noted the same sort of dark spots in the center of the old pustules when the entire pustule was removed intact by dissection. Fragments of the leafhopper egg shell were found in these dark spots.

DISCUSSION

The evidence just presented suggests that the pustular type of measles originally described by Hewitt and Truax is caused by the deposition of eggs of Typhlocyba pomaria⁵ in the tissues of apple twigs. Since the original specimens apparently are no longer in existence it is impossible to demonstrate that leafhopper eggs actually were deposited in the twigs from which Hewitt and Truax prepared their original description. However, this cause is indicated by the following circumstantial evidence: (1) Pustules similar to those described by Hewitt and Truax as the pustular stage of apple measles may be found on apple twigs in Arkansas orchards at the present (2) These pustules have the "burrowing tendency" and in the older stages have the "hollow core" described and illustrated by Hewitt and Truax in their original paper. (3) Leafhopper eggs or egg-shell fragments can be found in these pustules. (4) In microscopical sections of the pustules known to be the result of leafhopper egg-laying activities the histological changes are identical with those described and illustrated by Hewitt and Truax. (5) The pustular stage described by Hewitt and Truax is superficial, i.e., the histological changes involve the epidermis, phelloderm, and outer layers of the cortex. Their illustrations show a definite opening in the epidermis connected with the hollow core or central portion of the pustule. This is precisely the condition to be expected if the pustule was caused by a leafhopper. The egg was inserted through the opening and the nymph likewise emerged through the opening. (6) Hewitt and Truax noted that the bast fibers (pericyclic fibers) were sometimes involved. The same phenomenon may be observed in sections of pustules containing leafhopper eggs. (7) Hewitt and Truax were able to withdraw the entire pustule attached to the epidermis after proper treatment to soften the tissues. The pustules studied by the writers can be removed intact by dissection, leaving holes in the cortex similar to those pictured by Hewitt and Truax. (8) Hewitt and Truax's illustration of the underside of the pustules shows dark spots in the center of the cores. The same sort of spots, containing remnants of the egg shell, were noted in the pustules removed intact by the writers.

⁵ According to Ackerman and Isely (1) there are five species of leafhopper attacking the apple in northwest Arkansas, but only two of these species, namely, *Typhlocyba pomaria* and *Empoasca maligna*, deposit their eggs in the twigs. Of these *T. pomaria* is the only one numerous enough to be responsible for the symptoms discussed.

The similarity between the pustules described by Hewitt and Truax as a form of apple measles, and those caused by the egg-laying activities of Typhlocyba pomaria is sufficient, the writers consider, to prove that the two types of pustules are identical.

This being the case, it may be concluded that the pustular type of apple measles disease as originally described by Hewitt and Truax is not a "physiological trouble" but is caused by the egg-laying activities of the leafhopper Typhlocyba pomaria.

SUMMARY

Apple measles as originally described by Hewitt and Truax consisted of two types: the pustular and the scurfy.

The symptoms of the pustular type are distinctly different from those of the scurfy type and likewise are not identical with those of other obscure apple bark diseases described by later investigators.

A comparison of material collected in Arkansas in recent years with the original description and photographs (none of the original specimens being available) of the pustular type convinced the writers that this type still occurs in Arkansas orchards.

A study of these present-day specimens shows that the pustules are caused by the insertion of the winter eggs of the white apple leafhopper, Typhlocyba pomaria McAtee, in the twigs.

The histological features described and illustrated by Hewitt and Truax are found in apple twig tissues injured by leafhopper oviposition.

The writers conclude that the pustular type of measles, as originally described by Hewitt and Truax, is caused by the deposition of leafhopper eggs in the twigs.

ARKANSAS AGRICULTURAL EXPERIMENT STATION, FAYETTEVILLE, ARKANSAS.

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GENETIC LEAF ROLL OF IRISH POTATO SEEDLINGS¹

E. L. LECLERG²

(Accepted for publication June 15, 1945)

A leaf roll of small Irish potato seedlings grown from true seed in 3-inch pots was observed in the greenhouse during the winter of 1943. It was first observed in the progeny of a self-pollinated seedling (XL 72-1) which had been selected in Louisiana. The margins of the leaves of the affected plants rolled upward starting with the lower leaves and extending to the apical leaves (Fig. 1). Many of the rolled leaves had a red-purple tinge along the margins shortly after rolling became apparent.

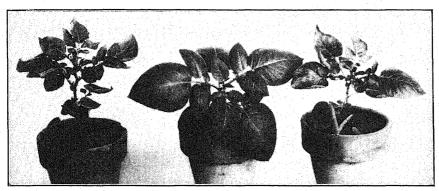


Fig. 1. Genetic leaf roll of potato seedlings. Normal plant in center.

Table 1 gives the number of segregates with leaf roll in progenies from crosses as well as in inbred progenies. It appears that 45 of 124 plants were rolled in the inbred progeny IL 12 (from XL 72–1 selfed). Leaf-rolled plants appeared in 4 out of 6 cross-progenies in which seedling XL 72–1 was used as a parent; however, in the other 2 cross-progenies (XL 225 and XL 237) no rolled plants occurred. The progeny XL 237 [(XL 72–1) × (XL 105–2)] consisted of 1,477 segregates, which is a number sufficiently large to indicate that some factor may be operating to inhibit expression of this character in the segregates of this progeny.

Stem-graft inoculations with these rolled plants on disease-free stocks all gave negative results, which indicates that the rolling was not caused by a virus. Tubers produced by plants subject to rolling were planted at Crossville, Tennessee, in the spring of 1944. None of the plants produced from these tubers showed any type of leaf rolling during the growing season.

¹ These investigations were conducted by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering; Agricultural Research Administration, United States Department of Agriculture, in cooperation with the Department of Horticultural Research of the Louisiana Agricultural Experiment Station.

² Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering; Agricultural Research Administration, United States Department of Agriculture; stationed at Baton Rouge, Louisiana.

Had the rolling of the leaves in the seedlings been caused by a virus the plants grown in the field from these tubers also should have been rolled; accordingly, the fact that no rolling occurred in the field-grown plants is further evidence that a virus is not associated with this type of leaf rolling. Furthermore, it is apparent from these results that this type of leaf roll is different from the nonvirus leaf roll previously described by the writer. The leaf roll herein discussed is limited to the small seedlings grown from true seed in clay pots and is not present in plants grown from tubers produced by these plants. The nonvirus leaf roll previously described, on the other hand, occurs only in field-grown plants and not in small seedlings.

TABLE 1.—Expression of genetic leaf roll in potato seedlings grown from true seed in the greenhouse at Baton Rouge, Louisiana, in 1943

	77. 71		Num	ber of
	Pedigree number	Parentage	Rolled plants	Nonrolled plants
<u> </u>	XL 225	Katahdin×(XL 72-1)	0	34
	XL 226	Sebec × Katahdin	0	28
	XL 227	$Katahdin \times Sebec$	0	25
	${ m XL}$ 228	Sebec \times (XL 72-1)	9	330
	XL 229	$(XL 72-1) \times Sebec$	8	287
	XL 230	$528-170 \times (XL 72-1)$	16	620
	${ m XL}$ 232	$(XL 72-1) \times 528-170$	5	172
	XL 233	$(XL 105-2) \times Katahdin$	0	925
	XL 235	$\dot{\mathbf{F}}$ riso \times Earlaine	0	17
	XL 236	$Friso \times Katahdin$	0	24
	XL 237	$(XL 72-1) \times (XL 105-2)$	0	1,477
	XL 238	$\dot{\mathbf{K}}$ atahdin \times 528–170	0	80
	XL 239	$528-170 \times \text{Katahdin}$	0	322
	XL 240	$(XL 105-2) \times 528-170$	0	1,273
	IL 10	Sebec selfed	0	60
	IL 11	528-170 selfed	0	752
	IL 12	XL 72-1 selfed	45	124
	$\overline{1L}$ $\overline{13}$	Katahdin selfed	0	133
	IL 14	Earlaine selfed	Ö	132

The results herein reported support the hypothesis that this type of leaf rolling is the result of the expression of a genetic factor.

Katahdin, Earlaine, and Sebec do not possess factors for this type of rolling since the character did not appear in inbred progenies of these varieties (Table 1). Likewise, seedling 528–170 did not have rolled plants in its inbred progeny.

It has been impossible to formulate a genetical factorial explanation of this character from the data in table 1. However, this type of leaf rolling is due to a recessive factor.

LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE, LOUISIANA

³ LeClerg, E. L. Nonvirus leaf roll of Irish potatoes. Amer. Potato Jour. 21: 5-13. 1944.

PRELIMINARY STUDIES ON PHYSIOLOGIC SPECIALIZATION IN TILLETIA TRITICI AND T. LEVIS IN CHINA¹

T. F. YU, H. R. WANG, C. T. FANG, AND S. Y. YIN²
(Accepted for publication June 14, 1945)

INTRODUCTION

Stinking smut is one of the most important diseases of wheat in North China. It is so serious that, in certain spring wheat growing regions, wheat has been replaced by barley. In South China, the disease seems less prevalent and destructive. Nevertheless epidemics of bunt have frequently been reported from certain localities.

The best method of reducing loss from stinking smut is the development of smut-resistant varieties. However, the problem of producing smut-resistant varieties is complicated by the existence of many physiologic races within *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kuhn. For this reason, varieties resistant to certain races may remain resistant only as long as they are exposed to infection by only such particular physiologic race or races of the pathogens. It seems, therefore, that in the course of developing stinking smut resistance in wheat, it is highly desirable to begin with studies on the number, prevalence, distribution, and pathogenic capability of physiologic races.

LITERATURE REVIEW

The first statement that specialization occurs in bunt was made by Faris (4), who, in the course of investigating the physiologic factors influencing infection by stinking smut organisms, obtained some preliminary evidence of differences in pathogenicity. Since then, specialization in both *Tilletia tritici* and *T. levis* has been studied by many investigators (1, 2, 3, 5 through 15), who have shown conclusively that these smut fungi comprise distinct physiologic races.

Because of the lack of uniformity in the use of differential hosts and in the system of numbering distinct races, many of the races identified and numbered by the various workers might be duplicates. In an attempt to work out a standard system for the identification of physiologic races of bunt organisms in the United States and Canada, Rodenhiser and Holton (16) have reinvestigated the races described by the previous workers and, in addition, have tested a number of collections of smut. They have selected 10 varieties of both winter and spring wheats as the standard differential hosts and were able to identify and number 10 races of *T. tritici* and *T. levis*.

¹ Paper No. 22 from Division of Plant Pathology, The Institute of Agricultural Research, National Tsing Hua University.

² The writers wish to express their appreciation to Prof. F. L. Tai for his encouragement throughout the investigation and his criticism of the manuscript and to Dr. E. C. Stakman for reading the manuscript.

They are also indebted, for bunt material, to Dr. C. S. Wang, Honan, Mr. T. L. Nien, Kweichow; and Dr. L. Ling and Mr. Y. S. Wu, Szechwan. Dr. H. A. Rodenhiser of United States Department of Agriculture very kindly supplied seed of the differential wheat varieties.

Recently, Holton and Rodenhiser (12) have described 5 new races of *T. tritici* and 2 of *T. levis*. In this study, Martin and White Odessa were added as differential hosts in the winter wheat group, while Mindum was omitted, as a differential host, from the spring wheat group.

MATERIALS AND METHODS

Collections of bunt were requested in 1938 and 1939 from many of the agricultural institutes in China. Forty-five collections were obtained for use as inoculum in the fall of 1938 and 12 more were obtained in the fall of 1939. Most of these collections used in the present studies were from single galls and were propagated on susceptible wheat. Every precaution was taken to keep them as pure as possible.

The smut reactions of 24 varieties of wheat were studied. Of these wheats 14 were either developed or introduced from foreign countries by the various agricultural experiment stations in China and the remaining 10 were those used by Rodenhiser and Holton for differentiating the physiologic races of the two species of *Tilletia* in the United States. Most of the Chinese wheats, with the exception of 2H80, which was one of Percival's wheats introduced into China, failed to differentiate the smut collections thus far obtained. Among the wheats selected by Rodenhiser and Holton as their differential hosts, Ulka and Hybrid 128 were susceptible and Ridit, Oro, Hussar, and Canus were resistant to all of the smut collections in China. Albit did not grow well, and Hohenheimer was discarded on account of its high degree of sterility under local conditions. Thus, only three varieties of wheat, Marquis, Mindum, and 2H80, finally were selected as differential hosts.

Seeds of differential hosts were treated with formaldehyde according to the standard method, thoroughly washed with water, and allowed to dry. The seeds and spores were then shaken together in an envelope until the seed was completely covered with spores. Three grams of inoculated seeds were planted in each 3-ft. row. Three rows constituted a plot, and plots were systematically arranged in 2 or 3 replications. Smut percentages obtained were based on counts of the total number of heads per plot. Three infection classes were used: Resistant class (R)—0-5 per cent infection; intermediate class (I)—6-20 per cent infection; and susceptible class (S)—21-100 per cent infection.

RESULTS

The results of 3 years' work are given in table 1. Reactions of the varieties were remarkably consistent. Ulka, which was used by Rodenhiser and Holton as the susceptible check, was also extremely susceptible to all of the smut races in China. Its smut reactions are in table 1 for comparison with reactions of the 3 differential hosts.

Nine of the 57 smut collections were Tilletia levis. On the basis of the percentages of infection they produced, they represent 6 distinct races.

Race 1 is the only race to which the 3 differential hosts are resistant. Race 2 is differentiated from race 1 by its capacity to infect Mindum, and race 6 by its development on Mindum and 2H80. Marquis has an intermediate reaction to race 3, and 2H80 has an intermediate reaction to race 4. Race 5 differs from race 4 by the intermediate reaction of Mindum and from race 3 by the intermediate reactions of both Mindum and 2H80.

A total of 48 collections of *Tilletia tritici* have been tested. On the basis of reactions of the same three differential hosts, these collections comprised 4 distinct races. All three differential hosts are resistant to race 1. Race 2 is differentiated from race 1 by the susceptible reaction of Mindum, race 3

TABLE 1.—Percentages of bunt obtained in 3 varieties of wheat exposed to 4 physiologic races of Tilletia tritici and 6 physiologic races of Tilletia levis at Kunming, China

•			P	ercent	agesa (of sm	itted :	heads	produc	ed by		
Host variety	Year tested	Ra	es of	T. tri	tici			R	aces of	T. le	vis	
		1	2	3	4		1	2	3	4	5	6
Marquis	1941 1942 1943 Av.	2.8 0.8 0.5 1.4	1.1 0.6 0.6 0.8	10.1 6.4 7.7 8.1	3.7 12.9 5.3 7.3		3.7 1.2 1.5 2.1	3.7 2.7 3.5 3.5	11.7 20.2 5.0 12.3	8.2 9.0 13.7 10.3	5.8 5.3 7.6 6.7	7.2 4.7 6.1 6.1
Mindum	1941 1942 1943 Av.	$0.9 \\ 1.1 \\ 0.0 \\ 0.7$	18.5 24.2 22.6 21.8	5.3 3.1 3.7 4.0	2.0 6.6 1.6 3.4		0.0 0.0 0.0	20.0 20.8 25.3 22.2	0.0 0.0 0.0	3.6 0.9 4.1 2.9	5.2 7.2 5.7 6.1	$11.2 \\ 8.4 \\ 9.0 \\ 6.2$
2H8O	1941 1942 1943 Av.	0.0 0.0 0.0	$0.4 \\ 0.0 \\ 0.0 \\ 0.1$	0.0 0.0 0.0	5.1 13.0 2.7 6.9		0.0 0.0 0.0	0.0 0.0 0.4 0.1	0.0 0.0 0.0 0.0	6.1 9.4 7.6 7.7	6.3 3.3 5.7 5.1	17.9 15.4 34.8 24.0
Ulka	1941 1942 1943 Av.	23.4 45.5 35.7 34.9	45.2 47.8 49.8 45.9	43.7 52.1 39.0 44.9	47.1 35.5 64.3 49.3		41.3 29.8 31.0 34.0	24.0 43.5 26.5 31.3	47.5 51.2 30.6 43.0	73.6 59.8 45.7 59.7	48.8 43.0 47.7 46.6	63.1 49.9 43.7 55.6

a Percentages for each year are averages from 3 or 4 plots.

by the intermediate reaction of Marquis, and race 4 by the intermediate reaction of 2H80. Table 2 and the keys show that the 6 races of *T. levis* and 4 races of *T. tritici* may be differentiated with the three varieties of wheat used.

Of the 9 smut collections obtained from North China, 7 were *Tilletia levis*. This probably indicates that *T. levis* is the predominant species in North China. Of the 48 smut collections made in South China, however, only 2 were identified as *T. levis*. Evidently, in South China *T. tritici* is predominant. The number of collections of *T. levis* was too small to permit a detailed analysis of the distribution of the species in China but the 45 collections of *T. tritici*, comprising 4 distinct races, may give a general idea of the distribution and prevalence of these races and of the species in South-

western China. The frequency of occurrence and the distribution by provinces and districts of the collections representing the 4 races of *T. tritici* are in table 3.

TABLE 2.—Reactions of 3 varieties of wheat which differentiate 6 physiologic races of Tilletia levis and 4 races of Tilletia tritici

Host	Race No. of T. tritici					Race No. of T. levis					
variety	1	2	3	4		1	2	3	4	5	6
Marquis	R R R	R S R	I R R	I R I		R R R	R S R	I R R	I R I	I I I	I I S

Race 1 of *Tilletia tritici* was collected more often than all other races and it occurred over wide areas in Southwestern China. Race 4 was collected only once in Kwangshung, Kweichow.

Key to 4 physiologic races of Tilletia tritici	
	Race No.
Marquis resistant	
Mindum resistant	1
Mindum susceptible	2
Marquis intermediate	
2H80 resistant	3
2H8O intermediate	
Key to 6 physiologic races of Tilletia levis	
	Race No.
Marquis resistant	
Mindum resistant	1
Mindum susceptible	2
Marquis intermediate	
Mindum resistant	
2H80 resistant	. 3
2H8O intermediate	. 4
Mindum intermediate	
2H8O intermediate	. 5

SUMMARY AND CONCLUSIONS

Three years' data on physiologic specialization in *Tilletia tritici* and *T. levis* in China have been obtained. Separation of races within each species was based on differences in pathogenicity on three varieties of wheat, Marquis, Mindum, and 2H80. Four races of *T. tritici* and 6 races of *T. levis* were identified and numbered T-1 to T-4 and L-1 to L-6 respectively.

In North China T. levis strongly predominates while in South China T. tritici is predominant. Race 1 of T. tritici is the most widely distributed in Southwestern China. It was found in 17 of the 18 districts from which collections were obtained. It was also the most frequently collected, comprising 35 of a total of 45 collections. This race, to which all the differential hosts are resistant, may be further separable into more races if the proper additional differential hosts are used.

In the northwestern corner of Yunnan, where bunt is usually epidemic, races 1, 2, and 3 of *Tilletia tritici* have been thus far found. The wheat

TABLE 3 .- Frequency of occurrence and distribution by provinces and districts of collections of 4 physiologic races of Tilletia tritici in Southwestern China

Province and district	No. of	Total				
Province and district	1.	2	3	4	- collec tions	
Tunnan						
Tali	2	1		******	3	
Hsihchow	1		2		3	
Tungchwan	2	1			3	
Feng-I	3				3	
Yang-pi	4	1	*****		5	
Tengschwan	$\tilde{2}$				2	
Total	14	3	2		19	
TOTAL	7.7	J	4		19	
Kweichow						
Ping-I	2	<u></u>	*****		2	
Ping-pa	3	******			3	
An-shun	3				3	
Tsingcheng	2				2	
Kwangshung	1			1	2	
Chengning		1			1	
Total	11	7		1	13	
10001	7.7		•••••		. 10	
Szechwan						
Sui-ning	2	2			4	
Si-chung	3	1			4	
Feihsien	2		*****		2	
Chingtang	ī	•••••	4000		$\overline{1}$	
Anhsien	$\bar{1}$				ī	
Santai	1				ī	
Total	- 10	3			13	
TO DOLL	10		*****		19	
Frand total	35	7	2	1	45	

variety 2H80 has been resistant to these 3 races. This wheat possesses, in addition to its smut resistance, many other desirable agronomic characteristics and will be valuable in breeding wheat resistant to stinking smut for this part of China.

INSTITUTE OF AGRICULTURAL RESEARCH, NATIONAL TSING HUA UNIVERSITY, KUNMING, CHINA.

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EPIDEMIOLOGY STUDIES ON STRIPE RUST OF WHEAT IN CHENGTU PLAIN, CHINA

LEE LING

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Stem, leaf, and stripe rusts are all of common occurrence on wheat in Szechuan province, China, but stripe rust is often the most important factor in the reduction of wheat yields. Although this rust is seldom absent, it varies considerably in severity from year to year. In Chengtu plain of this province, the rusts occur in the following sequence: (1) Puccinia triticina Erikss., (2) P. glumarum (Schmidt.) Erikss. and Henn., (3) P. triticina Erikss., (4) P. graminis Pers. In general, wheat is sown in that region in late October and harvested in the middle of May. Leaf rust appears on seedlings of early sown wheat about the middle of November, when the temperature averages not lower than 10° C. It is seldom seen when tempera-Stripe rust occurs in late November or early December tures are lower. and maintains itself without much spread until February of the following year, when it becomes epidemic if environmental conditions permit. average years, the stripe rust disappears in late April. Leaf rust reappears in late March to early April and becomes prevalent in May. Stem rust is the last to appear and gains in intensity in June, but it is too late to cause damage on wheat.

NATURAL HOSTS

Besides attacking wheat and barley, stripe rust occurs very commonly on Agropyron ciliare (Trin.) Franch. and A. semicostatum Nees (probably identical with A. caninum (L.) Beauv.) in Chengtu plain and its surrounding mountains. On these two grass hosts, however, the rust appears much later than on wheat, generally not until April, and it becomes prevalent in May. It seems to endure much higher temperatures than stripe rust on wheat. Seedlings of Bromus japonicus Thumb. have been observed infected with stripe rust, but never mature plants. Other wild grasses such as Bromus remotificrus Steud., B. tectorum L., and Elymus sibiricus Roch. have never been found infected. Rye is not cultivated in the plain region, but in the experimental plots it has not had stripe rust and has been seen rusted only by Puccinia dispersa Erikss. and Henn.

Since Newton and Johnson (12) and Becker and Hart (2) have found that stripe rust from Agropyron is capable of attacking wheat, the local Chinese collections from Agropyron ciliare were inoculated on the standard assortment of differential hosts to determine whether the physiologic races harbored on the grass host were identical with those on wheat. Two races were isolated from such collections. One attacked only two barley varieties, Fong Tien and Heils Franken, and produced scattered infections of type 2 instead of the usual stripe-like sori. The other attacked the two barleys

with type 3-4 infection, Petkuser rye with type 2 infection, and Chinese 166 wheat with type 3 infection. The latter race appears to be similar to 376A of Becker and Hart (2) from barley. It is also important to note that in 1941 the inoculations were made near the end of April and sori appeared in early May, a period during which the mean temperature was 24.7° C. At the same time several collections of rust from wheat were inoculated but failed to produce infections, apparently because of the high temperatures prevailing. Such results indicate that the grass is not likely to serve as a source of supply of urediospores for wheat during the unfavorable months in summer under local conditions.

EFFECT OF TEMPERATURE ON GERMINATION OF UREDIOSPORES

The cardinal temperatures for germination of urediospores of *Puccinia glumarum* have been studied by several investigators (7, 12, 14, 15). The minimum temperature is just above 0° C., the optimum ranges from 10° to 12° C., and the maximum from 23° to 27° C.

TABLE 1,—The effect of temperature on the germination of urediospores of Puccinia glumarum

Temperature.		Number spores	3	Germ	inated spo	resa		
in degrees C.			ed Number			Per cent		
4.5		635		72		11.3		
10		1,181		300		25.4		
11.5		1,048		287		27.4		
14		891		223		25.0		
16		1,055		222		21.0		
18		1,377		86		6.3		
21		1,530		22		1.5		
25		1,600		6		0.4		
28		2,000		0		0		
32		2,000		Ō		0		

a After 18 hours.

As stripe rust is known to be particularly sensitive to high temperatures, the knowledge regarding the influence of temperature on the germination of urediospores of local physiologic races would be helpful in explaining the seasonal occurrence of stripe rust as well as the development of epidemics in China. In experiments in 1940, we used a race prevalent on native wheat which is similar to race 31 of Straib (14) except that it produces a 4 infection type on Spaldings Prolific wheat. The urediospores were shaken from the open pustules of infected leaves onto plates of potato-dextrose agar. The plates were immediately placed in incubators or in the refrigerator and examined at intervals. The average results of two experiments are summarized in table 1. At the optimum temperature of 11.5° C. germination started in four hours, reached over 1 per cent after 6 hours, and reached 27 per cent after 18 hours. The maximum appeared to lie around 25° C. The figures in table 1 are the averages obtained in two experiments.

LONGEVITY OF UREDIOSPORES

The profound influence of environmental conditions on the viability of urediospores of stripe rust is well indicated by the divergent results given by different investigators. Hungerford (6) found that the urediospores may remain viable for at least 58 days at ordinary room temperature in Oregon, United States, and that only a small percentage germinated after 63 days in a desiccator. In England, Mehta (7) obtained 5 per cent germination in the laboratory and 15-20 per cent at 2.5° or 5° C. after the urediospores had been exposed for one month. Ducomet (4), in France, determined that the urediospores were able to germinate during a period of at least of 235 days after formation. Becker (1) reported the retention of germinability of urediospores for 433 days at optimum conditions of 0° C. and 40 per cent relative atmospheric humidity. Menacacci (11) recorded that the urediospores taken from June infection in the vicinity of Rome and placed indoors lost their germinability on the 22nd day. Raeder and Bever (13) proved that the optimum humidity and temperature for the retention of germinability of urediospores were 49 per cent and 9° to 13° C. respectively. Under such conditions, spores remained viable for 88 days. The upper thermal limit of endurance for the rust was studied by Butler and Hayman (3), who found that the urediospores when moist would not endure exposure at 45° C., for 5 minutes.

Since the stripe rust apparently depends entirely upon the uredial stage for its perpetuation, the longevity of urediospores under local conditions was studied. As the urediospores found between the glumes have been reported to retain their germinative power longer (11), both the infected leaves and heads were used as the source of urediospores in the experiments of 1940. The viability of urediospores was tested at intervals by germinating them on agar plates. The materials were kept in open vials and held in the following conditions: (1) Outdoors, (2) in the laboratory, (3) in a desiccator, (4) in an incubator at 32° C. While the experiments were made the room temperature was approximately 20° C. and relative humidity was high.

Rusted leaves of a very susceptible native variety of wheat were collected on April 23. The initial percentage of germination of urediospores was 10.6. On April 27, the germinative power had decreased only slightly, except for those spores kept at 32° C., only 1.7 per cent of which germinated. After April 27, the germinability declined rapidly. On May 14, 21 days after the beginning of the experiment, the germination of urediospores kept outdoors was 3.2 per cent, while germinability of those under other conditions had decreased to little more than 1 per cent. On May 27, very few spores germinated under all the conditions tested.

Urediospores between glumes of Blé rouge prolifique barbu were collected April 27, and 21 per cent germinated. On May 3, the percentages of germination were all above 12 except for spores kept at 32° C., only 1.4 per cent of which germinated. On May 14, seventeen days after collection,

the germination decreased to 2.4 per cent for the urediospores held outdoors, to about 1 per cent for those in the laboratory and in the desiccator, and to a trace for those at 32° C. On May 27, few spores, either outdoors or in the desiccator, germinated, while none germinated in other treatments.

The results were checked occasionally by inoculating the urediospores directly to wheat seedlings. In all cases, the infectivity could be retained no longer than one week. These results agree with the fact that during the work of determining physiologic races, the rust samples sent from nearby districts through the mails usually failed to produce infections.

In 1943, similar experiments were made with urediospores from infected leaves kept in ordinary room conditions and outdoors. Spore germination was rather erratic, but, in general, the longevity of urediospores was about 18 days only.

In 1940, the effect of a constant high temperature of 36° C., which might be encountered locally in the hot summer, on the viability of urediospores of stripe rust was studied. At such high temperature, spores remained viable for only two days.

The urediospores of stripe rust are short lived under natural conditions, indoors or outdoors, or when kept in a desiccator. In the years tested, urediospores from infected leaves retained viability no longer than one month, while the urediospores between glumes were even shorter lived. The urediospores rapidly perished at high temperatures.

OVERSUMMERING OF STRIPE RUST

In the Chengtu plain, the winter is ordinarily so mild that the temperature seldom drops below 0° C. Therefore the stripe rust urediospores and mycelium overwinter without difficulty. Between the wheat harvest in May and sowing in October, however, there are about five months, part of which is a hot and wet summer. In July and August, the rainfall is heavy and temperatures above 30° C. frequently persist for several days. The relative atmospheric humidity is usually above 80 per cent during the summer and fall. Under such conditions, urediospores are unable to live long, and neither volunteer wheat nor Agropyron has been observed infected. Accordingly, the means by which the rust bridges over the gap between wheat crops must be sought outside the plain.

The Chengtu plain is bordered at its western edge by mountains of varying height. The peaks and ranges northwest of the plain are from 5,000 to 7,000 meters high (Fig. 1). Between low and high altitudes there is well-marked "vertical zonation" of climate and vegetation. The limit of growth of wheat and barley is approximately 3,600 m. (16). In the subalpine zone (3,000–5,000 m.), winter wheat is cultivated and its growing season is prolonged with the increase of elevation. At higher levels in this zone, wheat is sown in September and harvested the next August. Thus from the plain up to the high mountains, wheat is seen all the year around. The gap between crops in the plain is well bridged over by the croppings at higher altitudes.

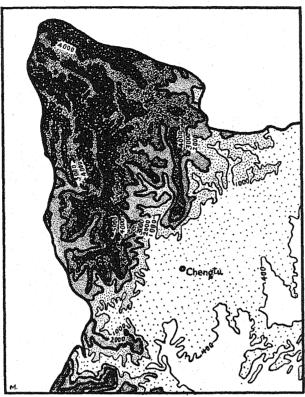


Fig. 1. Relief map of northwestern Szechuan Province, China.

In the plain, the stripe rust appears on wheat during May. Northwestward and in the mountain regions, the rust appears much later and may thrive throughout the summer. The monthly records of temperature in Sungpan (2,385 m.) as compared with those in Chengtu (503–576 m.) are in table 2. The rainfall on the mountains is always higher than in level country, which, in combination with lower summer temperatures, provides favorable conditions for rust development. Observations support such conclusions. At about 1,000 m. on Chineehnshan near Kwanhsien, stripe rust usually first appears in early April and is still seen in July on self sown wheat. In Lifan and Sungpan, at altitudes around 2,000 m., the stripe

TABLE 2 .- Average temperatures at Chengtu and Sungpan, Szechuan Province, China

Locality and altitude				Mea	n temperatu	re, in c	legrees	C.			
	Jan.	Feb.	Mar.	Apr.	May June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Chengtu (503- 576 m.)	5.9	8.4	12.5	17.3	22.1 24.9	26.8	26.2	22.0	18.1	12.2	8.1
Sungpan (2385 m.)	-1.7	- 0.3	4.0	7.8	10.6 12.9	16.1	16.0	12.1	8.0	2.3	-1.7

^a Averages for 1933-1943 at Chengtu and for 1937-1943 at Sungpan.

rust becomes prevalent in late June or July but is seldom seen in early August. In the spring wheat region on mountains near Sungpan, at altitudes slightly less than 3,000 m., the rust is not noticeable until the middle of July and it persists until harvest.

The evidence indicates that stripe rust survives the summer at higher altitudes. Above 2,500 m., there exists a gap of about one month between wheat crops. Since temperatures at altitudes above 2,000 m. seldom reach as high as April temperatures in the plain, there probably would be no difficulty for the urediospores to survive one month. Otherwise a quantity of urediospores from the spring wheat would supply sufficient inoculum for the fall crop at lower levels. According to the meteorological records of Sungpan, a northwest wind is not uncommon during October and November. The urediospores of the rust can be carried southeastward to the plain by the air current. In 1938, spore traps set in Chengtu first revealed the presence of urediospores of stripe rust in the air on November 28. Sporadic infections during the early season on wheat seedlings in the plain, which serve as the infection centers for further spread of the rust, also furnish evidence that inoculum is wind-borne. During April and May southeast winds and vertical movement of the air are frequent in the plain and doubtless facilitate the dissemination of urediospores to higher altitudes. The situation is comparable to that in India (8, 9, 10).

It is not known up to what levels the urediospores of stripe rust are able to survive the cold winter. According to Eriksson (5), they may endure -4.5° to -10° C. for 2 hours. But in the meteorological records of Sungpan (2,385 m.) temperature was as low as -17.6° C. in January, 1941. However, on the high mountains there are likely to be early snows, little alternate thawing and freezing, and late spring thaws. From the records on the tolerance for low temperatures by other cereal rusts, there is no reason to expect that stripe rust is unable to overwinter in its uredial stage or by dormant mycelium at least as high as where wheat grows.

INFLUENCE OF CLIMATIC FACTORS ON THE DEVELOPMENT OF EPIDEMICS OF STRIPE RUST

The prevalence of stripe rust on wheat has been investigated at Chengtu from 1938 to 1944 and its correlation with the meteorological factors has been attempted. In the seven years, the worst epidemic occurred in 1939. There was comparatively little rust in 1943 and 1944. The other years can be classed as intermediate in rust severity. In general, the winter in Chengtu plain is mild and fairly dry, but not rainless; yet winter drought is not usually suffered, because the humid atmosphere and the cloudiness reduce evaporation. Severe frosts are rare. In spring the rainfall is much increased and the temperature is higher. Such conditions permit the safe overwintering and early outbreak of the rust in average years. Meteorological data during the critical period of rust development are summarized in table 3. The situation of each year is given briefly as follows.

TABLE 3.-Meteorological records at Chengtu

$\mathbf{Y}\mathbf{e}\mathbf{a}\mathbf{r}$	Rainfal	l, in mm.		Relative humidity, in		Mean tem- perature,	Number of days of rain		
	Wintera	Spring	. :	$\operatorname{per \ cent} (\operatorname{FebApr.})$		in °C. (Apr.)	(FebApr.)		
1938	66.7	148.2		77.4	-	17.5	21		
1939	29.6	175.6		81.3		14.1	40		
1940	7.6	264.6		74.2		16.3	33		
1941	29.0	118.5		71.5		19.5	24		
1942	15.8	176.2		80.1		18.2	$\overline{32}$		
1943	13.0	91.1		77.2		16.8	$2\overline{4}$		
1944	21.7	106.8		76.2		17.5	38		

^a Winter refers to December of the previous year and January and February of the year indicated.

In 1938, an intermediate rust year, the especially wet winter and the high rainfall in February brought about an early outbreak of rust in the middle of February. But the spring was relatively dry in comparison with an average year, therefore the rust was unable to reach its maximum destructiveness in April and became almost unnoticeable toward the end of April.

In 1939, an epidemic year, the active development of rust occurred a little later than in 1938. Although the rainfall was not particularly high in the spring, it was well distributed. Several other factors were in extremes in comparison with other years: 1939 had the highest relative humidity, the highest number of rainy days, the lowest number of hours with sunshine, and the lowest mean temperature in April. The cloudy and wet weather brought about a very succulent growth of wheat, on which the water was amply retained. Moreover, the maturity of wheat as well as the appearance of the telial stage of rust was delayed by the low temperature in April. The uredial stage therefore had excellent chances for reproduction and there was a wide and rapid spread of the rust. Throughout the period the rust had an unusually thriving development until the temperature suddenly rose in early May. In the wheat fields the soil was covered by a yellow layer of spores for more than two months. This year was also very favorable to the growth of wheat, but many varieties failed to yield well because of the severe rust attack.

The intermediate rust year, 1940, was a year with weather conditions roughly contrary to 1938. The winter was very dry but the spring was wet. The active development of the rust was not noticed until early March. But the unusually high rainfall in March led to a severe outbreak. The destructiveness, however, was somewhat checked by the dryness in April, when the relative atmospheric humidity averaged 66.2 per cent. At the end of April and in May, there was again unusually high rainfall, which in combination with a relatively low temperature delayed the maturity of wheat even more than in 1939. The activity of stripe rust was resumed for a while in early May. This year was characterized by the prolonged period of rust development as well as by the highest yields for most varieties of wheat.

The very dry winter of 1941, an intermediate rust year, appeared to be responsible for the late outbreak of stripe rust, which did not start until early March, and probably was induced by considerable rainfall in late February. The progress of rust, however, was somewhat checked by dryness in March and the teliospores soon formed. Toward the end of March the recurrence of rains allowed the rust to become prevalent for a rather short period. This year was even more unfavorable to wheat growth than to the development of rust. The dryness in spring and the high temperatures in April caused a precocious growth and very poor yield in wheat.

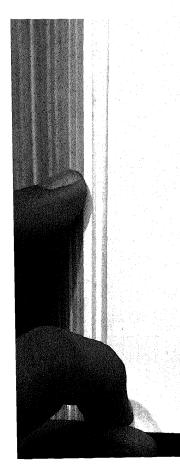
From December of 1941 to March of 1942 marks a period of drought, but the relative atmospheric humidity remained above 80 per cent. The year 1942 was an intermediate rust year. The rust started to gain in intensity only late in March. The unusually high rainfall in April, however, caused a sufficient retention of water both in the soil and in the plants to favor the development of the rust. This year was approximately intermediate among the seven years recorded in the prevalence of stripe rust, which was less destructive than in 1938, 1939, and 1940, but more destructive than in 1941, 1943, and 1944.

Both 1943 and 1944 were light rust years, the former being unfavorable to the growth of both wheat and stripe rust. Drought throughout the spring and winter kept down the rust, which was first noticed during mid-April. The rust situation of 1944 was comparable to that of 1943, though the dryness of the season was not so pronounced. The precipitation for 1944 was only slightly less than that for 1941 (Table 3), but its distribution varied. There was considerable rainfall in February, 1941, but much less in the same month of 1944. In both years March was dry, therefore the amount of precipitation in February would be important in determining the increase of actual inoculum early enough to initiate an epidemic at the right time.

From the foregoing discussions, it appears that the rainfall during the late winter and the spring is most important of all in affecting the prevalence of stripe rust in this region. However, not merely the amount of rainfall but its distribution as well should be taken into consideration. Rain should occur early in the season in order to initiate a rust epidemic. Besides its effect on the initiation of the disease, rainfall may render the wheat plants more susceptible to the rust. Both atmospheric humidity and cloudiness are effective in regulating the evaporation of water from plants as well as from soil. Low temperatures toward late spring favor repetition of the uredial stage and postpone the production of teliospores. These factors altogether have a profound influence on the increase of inoculum as well as the development of successive crops of urediospores during the growing season.

SUMMARY

In Chengtu plain, China, wheat rusts occur in the following sequence: (1) Puccinia triticina, (2) P. glumarum, (3) P. triticina, (4) P. graminis. The stripe rust caused by P. glumarum, however, is often the most important factor in reducing the yield of wheat.



Besides attacking wheat and barley, stripe rust occurs very commonly on *Agropyron ciliare* and *A. semicostatum* in the plain and surrounding mountains. On the grass hosts the rust appears later in the season than on wheat. Both the physiologic races isolated from *Agropyron* are different from those on wheat. Hence the grasses are not considered to have any importance in the epidemics of stripe rust on wheat.

The urediospores of stripe rust germinate best at 11.5° C. and the maximum lies around 25° C. Above 20° C. germination is much retarded and the final percentage is very low.

Under local conditions with high atmospheric humidity prevailing, the urediospores remain viable no longer than one month. The infectivity of spores often is retained a much shorter time, usually not more than one week. At high temperatures, the urediospores rapidly perish. At 36° C., the spores are viable for only two days.

In the plain the winter is so mild that stripe rust appears to have no difficulty in overwintering by means of either the urediospores or mycelium. Between crops of wheat, however, there exists a gap of five wet and hot summer months during which the stripe rust can hardly survive. On the mountains west of the plain, the season for winter wheat is much prolonged and spring wheat is cultivated at altitudes around 3,000 m. Stripe rust has been seen at high altitudes persisting on wheat throughout the summer. From those high levels, the urediospores are conveyed by wind southeastward into the lower altitudes to infect the fall sown wheat. The sources of inoculum for subsequent infections on wheat at higher altitudes are either the urediospores which have overwintered in situ or the urediospores blown back from the lower levels, or both.

The influence of climatic factors on the prevalence of stripe rust have been investigated from 1938 to 1944 at Chengtu. The results indicate that the amount and distribution of rainfall in late winter and spring are most important in determining the development of rust epidemics. Atmospheric humidity and cloudiness also are of significance because of their effects on the evaporation of water from the plants and from soil. Low temperatures in late spring delay the maturity of wheat and favor the repetition of the uredial stage of the rust. Among the seven years recorded, the worst stripe rust epidemic occurred in 1939, while there was very little rust in 1943 and in 1944. The other years are classed as intermediate.

University of Nanking, Chengtu, China.

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COPPER SULPHATE AS AN ERADICANT SPRAY FOR POWDERY MILDEWS

C. E. YARWOOD

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Powdery mildews are among the most easily controlled of fungus diseases of foliage. While a great variety of chemicals in liquid and dust form have been successfully used to control powdery mildews, sulphur dust was one of the first, and to this day remains the most universally successful. Liquid sprays containing lime-sulphur or wettable sulphur are, in general, similar in effectiveness to sulphur dust, but are more adhesive and more expensive to apply. Lime-sulphur spray is generally more injurious to the host and more effective as a fungus eradicant than either wettable sulphur or sulphur dust. For the relatively few cases where sulphur sprays or dusts are undesirable, one of several copper-containing sprays is usually used, and the writer feels that bluestone (copper sulphate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) with a spreader is one of the most efficient, perhaps the most, reported to date.

Radelyffe (6) in 1861 was perhaps the first to use bluestone as a foliage spray. He used 2 oz. bluestone to a stable bucket of water (probably between 0.3 and 0.6 per cent) and poured it on the rose bushes from a fine spout. He reported that the roses were cleaned of powdery mildew. He recorded no foliage injury but his statement that he would use a lower dosage next year indicated that injury may have occurred. In commenting on Radelyffe's method of mildew control, another writer (1) cautioned against the use of bluestone on foliage, but he reported no experiments or observations. Two years later Radelyffe (7) repeated his recommendation of bluestone for control of rose mildew, though he also gave sulphur as an acceptable treatment.

Following the discovery of Bordeaux mixture, Millardet (4) in France made extensive tests of bluestone sprays on foliage for the control of grape downy mildew, and Taft (8, 9) in Michigan recommended 1–1000 to 1–4000 bluestone sprays on fruit trees in foliage. These treatments have disappeared from present recommendations. Blake (2) used bluestone spray at the rate of "about a tablespoon to a bucket of water," and claimed perfect eradication of rose mildew. In spite of these favorable though fragmentary reports, and in the apparent absence of any definite contrary evidence, the use of bluestone spray was apparently not accepted by the public or by plant pathologists, and the writer is aware of no recommendation of the use of bluestone for the control of a powdery mildew at present. Soluble coppers such as copper ammonia mixtures and copper oleate in oil, and less soluble coppers such as Burgundy mixture, Bordeaux mixture, and cuprous oxide are being successfully used for the control of powdery mildews, but little quantitative information on their comparative value is available.

The experimental demonstration that the eradicant value of Bordeaux for bean powdery mildew decreased with increasing amounts of lime in the spray (14) stimulated this further study of bluestone as an eradicant spray for powdery mildew.

EFFECT OF A SPREADER ON THE SPRAY DEPOSIT ON FOLIAGE

Spray deposit was calculated from the measured wet deposit and the known fungicide content of the spray. If it can be assumed there is no selective adherence and that there is no physical change in the liquid induced by the suspended or dissolved fungicide, the deposit of any dosage of spray mixture could be calculated from the measured deposit of a similar appli-

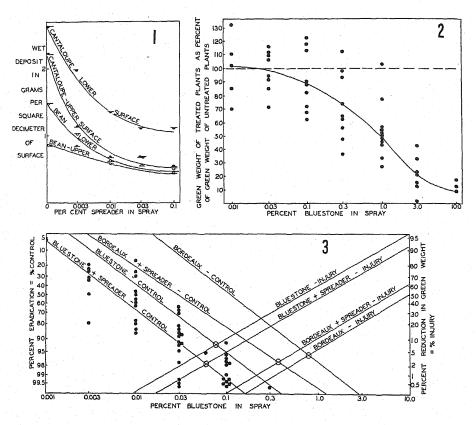


Fig. 1. The relation of concentration of spreader (glyceryl alkyl resin) in the spray to the wet deposit on the upper and lower surfaces of cantaloupe and bean leaves.

FIG. 2. The relation of concentration of bluestone in spray (with 0.05 per cent spreader in all cases) to injury to beans. Each dot represents the green weight of the leaves of young Pinto beans, grown in 4-inch pots in the greenhouse, 5 to 10 days after spraying with bluestone of the indicated concentration, expressed as a percentage of the green weight of similar unsprayed plants. The smooth curve approximates the average results.

Fig. 3. The relation of concentration of bluestone in spray to eradication of bean mildew and to injury of noninfected plants. Only the readings (dots) for bluestone plus spreader used as an eradicant are given. In all cases the straight line best fitting the average results is indicated. The Bordeaux mixture was prepared from equal parts of bluestone (CuSO₄· $5\rm H_2O$) and line (CaO), and the dosage is the percentage of bluestone in the final spray.

cation of water. While these assumptions are not justified under all conditions, their acceptance is believed to introduce no errors of consequence in the present study and data for water only will be presented here, though deposit data have been collected for lime-sulphur, Bordeaux, and Burgundy sprays. Weighed single leaves held in a vertical position were sprayed with a DeVilbiss atomizer with 35 lb. air pressure at a distance of about 10 inches until maximum deposit appeared to have been reached and runoff had just started and the leaf was again weighed. The leaf area was measured with a planimeter and the spray deposit was calculated as grams of wet spray per square decimeter of leaf surface. Glyceryl alkyl resin (B1956 spreader of Rohm and Haas Co.) was used as a spreader and the quantitative effect of spreader on deposit was determined. Average results of one test in triplicate are in figure 1. Water alone gave a deposit of 2.6 g. per sq. dcm. on the lower surfaces of cantaloupe leaves, which were poorly wetted but retained the water in large drops before runoff. Addition of 0.003, 0.01, and 0.03 per cent spreader improved the wetting of the leaves and reduced the deposit. With 0.03 per cent spreader there was apparently perfect wetting and a deposit of 1.2 g./dcm.2; and further addition of spreader had no apparent effect. Similar additions of spreader had similar relative effects on the deposit on the upper surfaces of cantaloupe leaves and on the upper and lower surfaces of bean leaves, though the actual deposit was lower on these A concentration of 0.05 per cent glyceryl alkyl resin was therefore arbitrarily chosen as the dosage to be used in tests where the addition of spreader was involved, and spraying was always continued until runoff had begun.

BLUESTONE INJURY TO FOLIAGE

Spray injury was quantitatively determined by spraying healthy plants with a series of dosages of the test spray and weighing the foliage after maximum injury had developed. This was usually 5-10 days after spraying. The reduction in green weight was a measure of spray injury. Detailed results of spray injury to beans from bluestone plus spreader in 11 tests in the greenhouse are in figure 2 and the data show considerable variation, but significant injury did not result until concentrations of 0.1 per cent bluestone and above were used. For convenient comparison with the data on eradication of bean powdery mildew, the average data on injury to bean foliage from bluestone with and without spreader and from Bordeaux with and without spreader are expressed in figure 3 as the nearest straight line on a logarithmic probability scale (10). To produce 5 per cent reduction in green weight required a spray dosage of 0.062 per cent bluestone without spreader, 0.11 per cent bluestone with spreader, 0.27 per cent Bordeaux with spreader, and 0.77 per cent Bordeaux without spreader. The greater injury from bluestone without spreader than from bluestone with spreader is probably due to the greater deposit without spreader (See figure 1), but the cause of the greater injury from Bordeaux with spreader than from Bordeaux without spreader is undetermined.

Plant injury was also estimated in some tests on a scale of 0 to 100 in which 0 indicated no injury and 100 indicated death of all leaves. Data from all tests of host injury with bluestone sprays with and without spreader are in table 1. With beans it was found that the rating and weighing methods gave similar results. Spray concentration for 50 per cent injury ranged from a minimum of 0.035 per cent bluestone without spreader for greenhouse-grown mustard to over 10 per cent bluestone for garden beet in the greenhouse. With most plants the addition of a spreader increased the dosage necessary for 50 per cent injury. With peas in the greenhouse, however, bluestone spray with spreader caused more injury than bluestone spray without spreader. This probably was because practically no wetting of pea

TABLE 1.—Foliage injury from bluestone spray

		No.	Method of	Dosage (per cent blue- stone in spray) for 50 per cent injury				
Plant	Location	of tests	estimating injury	Without spreader	With 0.05 per cent spreadera			
Bean	Greenhouse	11	weight	0.66	1.2			
do	do	3	rating	0.5	1			
do	Field	1	do	0.8	1.8			
Cucumber	Greenhouse	6	weight	0.5	1.0			
do	Field	1.	rating	0.3	0.8			
Cantaloupe	do	1	do	0.3	0.8			
Gardenbeet	Greenhouse	1	do	> 10	> 10			
Grape	Field	1.	do	3	4			
Pea	Greenhouse	2	weight	10	3			
do	Field	1	rating	1	2			
Нор	do	1	do	1.8	1.8			
Mustard	Greenhouse	2	weight	0.035	0.15			
Potato	do	1	rating	*******	1.5			
Tomato	do	2	do	*******	1.5			
Rose	Field	1	do	1	1			
Apple	do	1	do		> 0.3			

a Glyceryl alkyl resin.

leaves occurred unless a spreader was added. With increasing amounts of spreader (0.05, 0.15, and 0.45 per cent) added to bluestone solution, injury to peas in the greenhouse was progressively increased. With peas in the field, however, where the foliage was less difficult to wet, injury was apparently greater without a spreader than with one. Foliage injury from bluestone sprays is greater when the spray dries slowly than when it dries rapidly, is greater from applications to the lower leaf surface than from applications to the upper leaf surface, and is greater on mildew-infected than on healthy plants. Many other factors are probably important in determining the severity of leaf injury from bluestone. On one occasion (Dec. 31, 1944) greater injury to greenhouse beans resulted from an application of 1 per cent Bordeaux than from 1 per cent bluestone, and in several tests greater injury has resulted from Bordeaux without spreader than from Bordeaux with spreader.

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ERADICATION OF POWDERY MILDEWS

The infected plants were sprayed with the test fungicide, and the amount of living mildew on check and treated plants was estimated 5 to 10 days after spraying on an arbitrary rating scale of 0 to 10 in which 0 indicated no mildew present and 10 that the leaf surface was entirely covered. The mildew present before spraying, but killed by the spray treatment, was usually still apparent on the leaves at time of recording the incidence of mildew, but was easily distinguished from the living mildew and was not included in the record. In some tests the mildew colonies were counted on unit areas and the counting method gave results parallel to the rating method; but a lower dosage of inoculum was necessary for the counting method because with heavy inoculation the powdery mildew colonies merged and could not be individually distinguished with the unaided eye by the time infection became evident. Upper and lower leaf surfaces were rated separately. Control was calculated by expressing the difference in rating between treated and check plants as a percentage of the rating for the checks.

Average results, presented as the best straight line fitting the data on a logarithmic probability scale (10) for greenhouse tests of the eradication of bean powdery mildew where a range of concentrations of bluestone and Bordeaux with and without spreader was applied 4 to 8 days after inoculation are in figure 3 and the detailed values for bluestone plus spreader are indicated. In this figure are also given the data on injury to healthy beans by these same sprays. Since the four curves for eradication are apparently approximately parallel, and the four curves for injury are also approximately parallel, all materials can be conveniently compared for eradication and injury; and the intersection points for eradication and injury by the same materials, indicated as circles, offer a good basis of overall comparison of the four sprays studied here. Bluestone plus spreader required a spray dosage of only 0.042 per cent bluestone for 95 per cent control by eradication while bluestone without spreader, Bordeaux plus spreader, and Bordeaux without spreader required about 2.5, 6, and 18 times this dosage, respectively, for equivalent control under these conditions. It is, of course, the balance between plant injury and disease incidence (as affected by eradication in this case) which is the best index of merit of a material, and which determines the better of a group of fungicides. For results as presented in figure 3, the lower the level of the intersection point of the control and injury curves, the lower the total of disease plus injury at that and similar dosages: and if low percentage disease and low percentage injury are of equal quantitative merit, the optimum dosage will be indicated by the point of intersection of the control and injury curves, disregarding such factors as cost On this basis as well as on the basis of the lowest dosage for control, bluestone plus spreader was the most effective of the four sprays compared. The optimum concentration of bluestone plus spreader was 0.06 per cent bluestone which was associated with 2 per cent disease and 2 per cent injury, the optimum concentration of bluestone without spreader was 0.08 per cent bluestone which was associated with 8 per cent disease and 8 per cent injury, the optimum concentration of Bordeaux plus spreader was 0.4 per cent Bordeaux which was associated with 3 per cent disease and 3 per cent injury, and the optimum concentration of Bordeaux without spreader was 0.8 per cent Bordeaux which was associated with 4 per cent disease and 4 per cent injury. Increasing the dosages above these would decrease the disease (or increase the control by eradication) but the increase in injury would be greater than the decrease in disease. For example, 0.1 per cent bluestone with spreader would give about 0.7 per cent disease and 5 per cent injury, or a total of 5.7 per cent disease plus injury, while at the optimum dosage of 0.06 per cent bluestone plus spreader the disease plus injury was only about 4.8 per cent. The specified values are not sufficiently significant that differences of such small magnitude due to spray dosage can be expressed with any degree of certainty, but the principle would appear valid for this or smaller or greater differences.

Fewer and less detailed studies of bluestone plus spreader as an eradicant spray have been made on the powdery mildews of cucumber, cantaloupe, mustard, grape, rose, peach, and apple, and in all cases approximately perfect eradication of powdery mildews was secured with approximately 0.1 per cent bluestone plus spreader.

With rose powdery mildew, plants of the varieties Night, Crimson Glory, and Rose Picture, grown 8 feet apart in a field plot at Albany, California, were sprayed or dusted May 10, May 29, June 21, and July 12, 1944, with 0.01 per cent bluestone plus spreader, 0.03 per cent bluestone plus spreader, 0.1 per cent bluestone plus spreader, 1 per cent lime-sulphur plus spreader, and sulphur dust, in a non-replicated series, and 4 checks of each variety were maintained throughout the plot. Mildew severity was rated May 15, July 3, and July 17, and 99.8, 100, 100, 97.3, and 100 per cent control, respectively, resulted from these treatments. Mildew was present, but not severe, on all check plants. There was no plant injury apparent from sulphur dust or 0.01 per cent bluestone, but 0.03 per cent bluestone caused slight injury noticeable on Rose Picture only, while 0.1 per cent bluestone caused injury estimated at about 10 per cent on all varieties. Even this amount of injury could easily have been overlooked.

OTHER FORMS OF COPPER AS ERADICANT SPRAYS

Sprays containing the soluble coppers, copper sulphate, copper nitrate, copper chloride, copper acetate, and copper-ammonia mixtures have been compared with sprays containing the relatively insoluble coppers, Bordeaux, Burgundy, cuprous oxide, copper phosphate, copper carbonate, and copper arsenite for the eradication of bean powdery mildew in the greenhouse. In general, the soluble coppers were eradicant at lower dosages than the insoluble coppers, and copper nitrate, copper chloride, and copper acetate were similar in effectiveness to copper sulphate. Burgundy mixtures containing one and one-half parts of sodium carbonate for each part of bluestone were

effective at lower dosages than the same concentrations of copper as Bordeaux containing equal amounts of bluestone and lime. With varying bluestone: lime ratios the eradicant value of Bordeaux decreased as the amount of lime in the spray increased.

NON-TOXICITY OF BLUESTONE TO POWDERY MILDEW CONIDIA

Conidia of Erysiphe polygoni from bean and from mustard dusted on the surface of 10 per cent copper sulphate solution germinated to the extent of 17 and 28 per cent, respectively, when controls on water germinated to the extent of 28 and 46 per cent, respectively, in the average of two tests. The conidia remained on the surface of the liquid and were not readily wetted and the germ tubes frequently came in contact with the bluestone solution without apparent injury, except that they were somewhat shorter than on water. When wetting agents were added to bluestone solutions of similar or lower strength, germination of conidia of bean mildew was prevented, but the wetting agent alone in water inhibited germination, and it was not determined to what extent the inhibition of germination was due to bluestone or to spreader. However, in view of these results the writer feels that with powdery mildews, as with downy mildews (12) and rusts (14), tests of the effect of chemicals on the germination of spores on glass are of relatively little value in appraising chemicals for the control of these diseases.

EFFECT OF AGE OF INFECTION ON THE SUSCEPTIBILITY OF POWDERY MILDEWS TO ERADICATION BY BLUESTONE SPRAY

Age of infection is defined for purposes of this paper as time from inoculation. Beans and cucumbers artificially inoculated with their respective powdery mildews were sprayed with a range of concentrations of bluestone plus spreader at various intervals. For each concentration series the concentration of bluestone for 95 per cent eradication was determined by plotting the observed results on a logarithmic probability scale (10). The LD95 (dosage for 95 per cent control) for different ages of infection, as determined in 5 tests with bean powdery mildew and in 3 tests with cucumber powdery mildew (Fig. 4), decreased from about 0.3 per cent bluestone with spreader at 2 hours (0 days) to about 0.03 per cent at 8 days from inoculation. This increasing sensitivity of powdery mildews to bluestone as the age of infection is increased is the opposite of what was expected, but might be considered in accord with the results on spore germination in vitro already presented, since it has been shown that bluestone was relatively non-toxic to germinating conidia.

Results of three tests of the effect of age of infection on the LD95 for lime-sulphur spray performed concurrently with the bluestone are also presented in figure 4. According to these data bean powdery mildew becomes increasingly resistant to lime-sulphur as the infections become older, or the tendency is the opposite to that with bluestone. The results with lime-sulphur cannot be considered conclusive, however, since this tendency was not



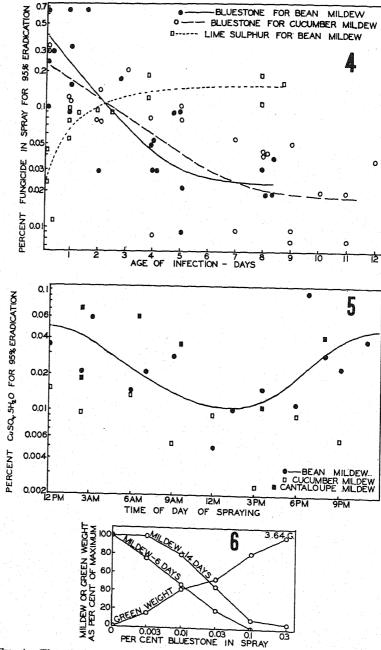


Fig. 4. The relation of age of infection (days after inoculation) to the dosage required for 95 per cent eradication of bean and cucumber powdery mildews with bluestone spray plus spreader, and for bean powdery mildew with lime-sulphur spray plus spreader. (with 0.05 per cent spreader) required for 95 per cent eradication of bean powdery mildew, and cantaloupe powdery mildew.

Fig. 6. The relation of concentration of bluestone spray (with 0.05 per cent spreader) to eradication of bean powdery mildew and to the green weight of the same plants.

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corroborated in two other tests, not run concurrently with the tests of bluestone.

EFFECT OF TIME OF DAY WHEN SPRAYS ARE APPLIED

Results of tests to determine the effect of time of day of spraying on the eradicant value of bluestone plus spreader applied 4 to 8 days after inoculation in 3 greenhouse tests with bean powdery mildew, 2 tests with cucumber powdery mildew, and 3 tests with cantaloupe powdery mildew are in figure 5. The average LD95 for bluestone with spreader for bean powdery mildew, presented as a smooth curve, ranged from a maximum of 0.05 per cent bluestone at 12 p.m. to a minimum of about 0.01 per cent at 12 noon. The limited number of tests, the variability of the data, and the apparently higher LD95 values secured in the tests summarized in figure 3 should be emphasized in considering the reliability of this apparent diurnal variation in the susceptibility of bean mildew to eradication by bluestone. In 2 tests each with cucumber powdery mildew and cantaloupe powdery mildew, tendency toward diurnal difference was not clearly apparent.

LOW PROTECTIVE ACTION OF COPPER SPRAYS

Most copper sprays or dusts tested to date have had little protective action against the powdery mildews studied, while sulphur sprays and dusts have had marked protective as well as eradicant action. Evidence of the greater eradicant than protective action of copper sprays and even of the stimulation of bean powdery mildew by protective applications of Bordeaux mixture has already been presented (13), but further data on protection derived from copper sprays will be presented here.

Results secured in protection spray tests with powdery mildews depend on the method of inoculation to a much greater extent than do results in eradication tests. The method of inoculation adopted as standard for tests in the greenhouse has been spraying of the test plants in the afternoon with a water suspension of powdery mildew conidia. On plants that had not received protective applications of fungicides, this method of inoculation has yielded heavier and more uniform infection of upper and lower leaf surfaces than any other method, though inoculation by dusting conidia direct from infected to healthy plants has usually resulted in heavier infection on the upper leaf surface, especially on plants treated with a protective fungicide. Powdery mildew conidia are normally and successfully disseminated in nature by being blown by air currents from a dry infected surface to a dry noninfected surface, therefore, inoculation by spraying plants with a spore suspension is unnatural. Furthermore, water is directly injurious to powdery mildews (11), especially at high impact pressures, and copper fungicides may be more toxic to powdery mildews in the presence of free water than in its absence (13).

Despite the admitted objections to the method, most studies of protective and eradicant sprays for powdery mildews have involved spraying with a spore suspension as a method of inoculation. In 6 closely comparable tests, the LD95 for bluestone plus spreader against bean powdery mildew was about 3 per cent bluestone for protection (determined by extrapolation, since bluestone caused so severe host injury that protection was difficult to determine at dosages above one per cent) and about 0.03 per cent bluestone for eradication; or the dosage for equivalent control by eradication was only about one one-hundredth that required for protection. In similar tests the LD95 for Bordeaux plus spreader was about 1.5 per cent for protection, and 0.4 per cent for eradication. Bluestone without spreader, Bordeaux without spreader, Burgundy mixture, cuprous oxide, and lime-sulphur have also been better as eradicant than as protective sprays though in no case was the difference as great as with bluestone. Emulsified cottonseed oil (tested in 2 paired comparisons at 3 dosages only) is the only spray tested which has given greater control by protection than by eradication. Tests of the same materials for cucumber powdery mildew indicate that with this disease also sprays are usually more effective as eradicants than as protectives.

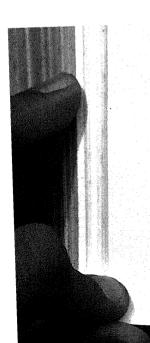
When inoculation has been by dusting the sprayed plants with dry powdery mildew conidia, control by protective sprays has been even less than when inoculation was by spraying with a spore suspension.

INCREASE IN GREEN WEIGHT AND YIELD OF SPRAYED PLANTS

The relation of dosage of bluestone spray to control of powdery mildew and to green weight and yield of plants has been measured in greenhouse and field tests with bean powdery mildew, cucumber powdery mildew, and cantaloupe powdery mildew. Results of some representative tests are given in figures 6–9. In the test represented in figure 6, duplicate 4-inch pots of two bean plants each were inoculated with powdery mildew on January 7, ten days from seeding on December 28.

On January 14, 7 days from inoculation, the growing point of each bean plant, that is, the growth beyond the inoculated primary true leaves, was removed, and the plants were sprayed with a series of concentrations of bluestone plus 0.05 per cent spreader. On January 20, 6 days after spraying and 14 days after inoculation, the severity of mildew on the upper and lower surfaces of the leaves was rated, and the average results are in figure 6 as the curve "mildew-6 days." Eight days later (mildew-14 days) mildew had increased considerably on all sprayed plants. The mildew on the plants treated with 0.1 and 0.3 per cent bluestone was probably due to reinfection after spraying. On January 31, 17 days after spraying, the new growth (axillary shoots) which had formed in the axils of the two primary true leaves since spraying was cut off and weighed, and it is indicated in figure 6 as green weight. This green weight varied in a fairly straight line relation from 0 grams on the similarly decapitated check plants to 3.64 grams on the plants sprayed with 0.3 per cent bluestone. At this time the primary leaves of the check plants and those sprayed with 0.003 per cent bluestone, all heavily infected, were dead.

In another similar test, beans seeded in the greenhouse, February 10, and inoculated February 21, were shaken occasionally to secure a fairly heavy



reinfection of the new growth. On February 29 the growing points of the plants were removed and the plants, in duplicate pots, were sprayed on February 29, March 6, March 26, and April 7 with spreader only, 0.001 per cent bluestone plus spreader, 0.003 per cent bluestone plus spreader, 0.01 per cent bluestone plus spreader, 0.03 per cent bluestone plus spreader, 0.1 per cent bluestone plus spreader, and 0.3 per cent bluestone plus spreader. The controls were unsprayed. On April 19, all leaves on the unsprayed plants were dead and the green weight of leaves plus fruit of the sprayed plants increased from 5.9 g. per plant for the plants sprayed with spreader only to 31.5 g. for plants sprayed with 0.03 per cent bluestone, but was only 15.5 g. for plants sprayed with 0.3 per cent bluestone. Records of mildew infection showed a progressive reduction in mildew with increasing concentration up to 0.1 per cent bluestone, which gave perfect eradication under these conditions.

The procedure of removing the growing point of each plant soon after inoculation, as in the two tests just reported, usually results in differences in green weight yield due to mildew infection and due to control of mildew which are greater and appear sooner than in non-decapitated plants, though differences of similar direction but lower magnitude resulted from the use of non-decapitated plants.

While bean powdery mildew apparently can be easily and thoroughly controlled by the use of bluestone spray in the greenhouse and in the field, this spray treatment gives no better control and is more laborious to apply than the standard dusting with sulphur. Bean powdery mildew lends itself more easily to intensive greenhouse study than any other powdery mildew known to the writer, and it is believed that results secured with this disease will apply in large part to many other powdery mildews, some of which cannot be commercially controlled with sulphur dust. Such a disease is cantaloupe powdery mildew, because cantaloupes may be severely injured by sulphur dust (5) as well as sulphur-containing sprays. Data of a comparative test of lime-sulphur and bluestone sprays for the control of cantaloupe powdery mildew in the greenhouse are in figure 7. Cantaloupes seeded in 4-inch pots December 20 were placed under conditions of moderately heavy infection from older infected plants, 6 replications were sprayed with each dosage of lime-sulphur and bluestone (plus 0.05 per cent spreader in all cases) on January 20, February 5, and February 25, and the green weight of the leaves was determined on March 11. For the dosages chosen, approximately equivalent mildew eradication was secured when bluestone was compared with lime-sulphur of about 10 times the dosage. With bluestone the green weight of the plants increased with increasing bluestone dosage and decreasing mildew up to 0.03 per cent bluestone, but then fell at 0.1 per cent bluestone. With lime-sulphur on the other hand, yield increased with 0.1 per cent lime-sulphur but was less with applications of 0.3 and 1 per cent lime-sulphur because of plant injury (Fig. 7).

In field tests, differences in mildew and in yield due to spray treatment have been less than in greenhouse tests. Kentucky Wonder beans seeded in

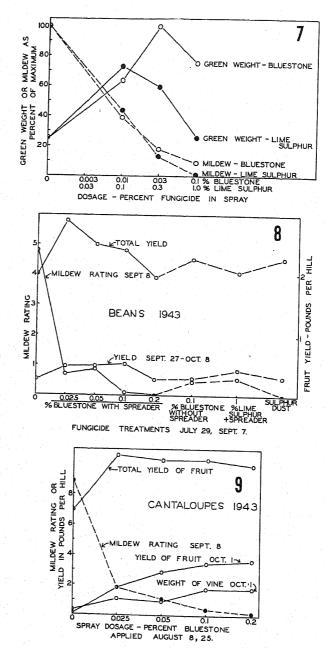


Fig. 7. The relation of concentration of bluestone spray plus spreader and of limesulphur spray (32° Baume) plus spreader to powdery mildew control and green weight in

greenhouse test with cantaloupes.

Fig. 8. The relation of spray material to powdery mildew infection and yield of Kentucky Wonder beans in field plots.

Fig. 9. The relation of dosage of bluestone spray plus spreader to powdery mildew control and to yield of No. 45 cantaloupes in field plots.

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hills May 6 were inoculated occasionally by dusting lightly with conidia from greenhouse plants and were sprayed in 7 replications on July 29 and September 7 with the materials indicated in figure 8. Mildew did not become severe, but marked control was secured with all treatments, though perfect control was secured only with 0.2 per cent bluestone plus spreader and with sulphur dust. Yields of green beans were recorded August 19, September 3, 13, and 27, and October 8. The highest total yield was from plants sprayed with 0.025 per cent bluestone plus spreader, but 0.025, 0.05, and 0.1 per cent bluestone all resulted in a higher yield than the check, and the relative increase was greatest in the last two pickings on September 27 and October 8.

With cantaloupe, previous tests had adequately demonstrated the danger of severe host injury from standard sulphur dusts and sprays and only bluestone (with 0.05 per cent spreader) was used in the 1943 tests. Cantaloupes seeded May 6 were artificially inoculated several times and were sprayed in 5 replicates of each treatment on August 8 and August 25. Mildew was severe and sprays containing 0.025 to 0.2 per cent bluestone gave marked control (see figure 9) which increased with dosage but was not perfect even with 0.2 per cent bluestone. Fruit yield was determined on September 3, 13, and 23, and October 1. Bluestone apparently delayed maturity and, on September 3 and 13, the fruit yield was greater on the check than on the treated plots (data not given in figure 9). The total yield, however, was greater for treated than for check plots. The most striking effect of spraying on yield was apparent at the final harvest on October 1, when the yield per hill ranged in a smooth curve from 0 for the control vines, which were mostly dead, to 3.52 lb. per hill for the vines treated with 0.2 per cent bluestone.

Field tests with cucumber powdery mildew in 1943 gave results similar to those presented for cantaloupe, except that the disease was not so severe and the yield increase from the treatments was less than in the case of cantaloupes.

DISCUSSION

Some explanation should be offered why a method of powdery mildew control reported in 1861 and neglected for 80 years should be revived in 1945. In the first place no one seems to have tried Radclyffe's bluestone treatment adequately and reported results, and Radclyffe's procedure was in part discredited by the warning of an anonymous writer as well as by the general knowledge of the injurious action of bluestone on foliage. Radclyffe's dosage, though not accurately stated, was much too strong. Also, his method of application was inefficient and his mixture lacked a spreader. The absence of a spreader in bluestone spray made it the poorest of the four spray mixtures compared in the present work (Fig. 3). Most important of all, perhaps, is the fact that sulphur dust for the control of powdery mildew was becoming well known in Radclyffe's time and, on plants tolerant to sulphur, no more efficient fungicide is yet known.

The suggestion in this paper that bluestone plus spreader is an effective practical control for several powdery mildews is limited to those cases where

sulphur dust or lime-sulphur are injurious to the host or give inadequate control. The best example of limitation of the use of sulphur because of host injury is cantaloupe powdery mildew (5), but there are undoubtedly others. No examples of failure to control powdery mildews with sulphur dust are known to the writer with certainty, but many have been indicated by observers. Such is indicated by Jacob (3) for established infections of grape powdery mildew and he recommends potassium permanganate plus here, bluestone plus spreader has proved a more effective eradicant for powdery mildews than the permanganate mixture. Many other spray materials, water alone, sea water, and solutions or suspensions of sodium carbonate, sodium bicarbonate, potassium hydroxide, nitric acid, sulphuric acid, sodium thiosulphate, ferrous sulphate, mercuric chloride, sodium silicate, zinc sulphate, vegetable, animal, and mineral oils, soaps, and materials used as spreaders have been tested, but none has been as effective at as low concentrations as bluestone plus spreader. Bluestone plus spreader has relatively little protective action, but effective practical control seems to be possible from its eradicant action alone.

SUMMARY

Addition of spreader to water sprays decreased the deposit but increased the coverage on cantaloupe and bean leaves. Wet spray deposit on lower leaf surfaces of cantaloupe decreased from about 2.6 g. per sq. dcm. when sprayed without spreader to 1.25 g. per sq. dcm. when spreader was added to the spray; and on lower leaf surfaces of bean the corresponding decrease was from 1.5 g. to 0.5 g. per sq. dcm.

Injury from bluestone sprays was measured on field- or greenhouse-grown bean, cucumber, cantaloupe, beet, grape, pea, hop, mustard, potato, tomato, rose, and apple foliage. The concentration of bluestone spray to cause 50 per cent injury varied from a minimum of 0.035 per cent bluestone for mustard to a maximum of over 10 per cent bluestone with beet. Bluestone injury was usually less when a spreader was added to the spray, but Bordeaux injury to greenhouse-grown beans was greater when a spreader was added. A 50 per cent injury to bean required about 10 times as much copper in the form of Bordeaux as in the form of bluestone.

Eradication of bean powdery mildew was secured at lowest copper dosages with bluestone plus spreader. For 95 per cent eradication a spray containing about 0.04 per cent bluestone plus spreader was required, while similar control required about 2.4 times as much copper in the form of bluestone without spreader, 6.4 times as much in the form of Bordeaux plus spreader, and 18 times as much in the form of Bordeaux without spreader. On the basis of maximum control with minimum injury, 0.06 per cent bluestone plus spreader was the most effective spray, followed in order by 0.4 per cent Bordeaux plus spreader, 0.8 per cent Bordeaux without spreader, and 0.08 per cent bluestone without spreader. The other soluble coppers (copper chloride, copper nitrate, and copper acetate) appeared to be about equal to

copper sulphate as eradicant sprays, but all insoluble coppers including Bordeaux, Burgundy, cuprous oxide, basic copper sulphate, copper oxychloride, and copper carbonate were distinctly less effective. Bluestone plus spreader was also superior to several other non-sulphur and non-copper chemicals tested.

Conidia of Erysiphe polygoni from bean and from mustard germinated well on the surface of solutions containing 10 per cent bluestone.

The spray concentration necessary for 95 per cent eradication of bean powdery mildew and cucumber powdery mildew decreased from a maximum of about 0.3 per cent bluestone applied at time of inoculation to about 0.03 per cent bluestone applied 8 days after inoculation. Eradication of bean mildew was secured from lower concentrations of bluestone spray applied during the day than from applications at night.

Heavier dosages of most sprays were necessary for protection than for eradication. For 95 per cent control about 100 times as much bluestone was necessary in a protective application as in an eradicant application.

The green weight of foliage and the yield of fruit on bean, cucumber, and cantaloupe plants on which powdery mildew was controlled with eradicant applications of bluestone plus spreader in greenhouse and field tests was greater than on similar unsprayed plants.

DIVISION OF PLANT PATHOLOGY. UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA.

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TIP BURN OF SUGAR BEET WITH SPECIAL REFERENCE TO SOME LIGHT AND NITROGEN RELATIONS

J. M. FIFE AND EUBANKS CARSNER 1

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Tip burn of sugar beet is a disease believed due to excessive accumulation of a substance or substances normally present in the plant. It has not been so diagnosed previously. Boncquet (1), in a paper concerned chiefly with curly top, described it incidentally and briefly under the designation "black edge" or "black tip." He at first thought it one of the types or stages of curly top, but later concluded that it was not. Robbins (2), in a study of sugar-beet mosaic, gave a somewhat more extensive description of tip burn which he regarded as an aspect of the mosaic.

DESCRIPTION OF SYMPTOMS

The symptoms of tip burn on vegetative beets in the field are distortion of the blades and death of more or less of the edge tissue often including the tip. The leaf blades may be cupped downward or in some plants the margins may be drawn in an upward curl. Frequently the leaf tissue between the large lateral veins becomes puffy and the blades then have a corrugated appearance. Much of the malformation of leaf blades appears to result from a greater retardation of growth of the vascular system than of the mesophyll tissue. The necrotic edges are tightly stretched. Often the tissue adjacent to the dark brown or black edges is yellow. These symptoms are usually on mature or nearly mature leaves. Occasionally, on plants where some of the larger leaves have these symptoms, the younger leaves may have severely truncated blades with blackened edges or even merely petioles with blackened tips (Fig. 1).

Affected beet plants with the characteristic downward cupping and necrosis on the edges of intermediate-aged leaves often have other symptoms that manifest early phases of tip burn (Fig. 2). Leaves younger than those with the conspicuous cupping may be blanched a yellowish green, with inconspicuous brown flecks, at the edges and tips. The marginal tissue so affected later dies and turns black. Development of these and other symptoms of tip burn seems to depend mainly on the duration of the conducive conditions.

Mother beets, especially when grown for seed in the greenhouse during winter, may have severe symptoms of types noted for vegetative beets and, in addition, the tips of the terminal or apical flowering shoot often blacken and die (Fig. 2).

Complete recovery is a regular characteristic of tip burn. The sub¹ Biochemist and Senior Pathologist, respectively, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

sequently developed leaves in vegetative beets or leaves and axillary shoots in flowering beets are entirely normal (Fig. 3).

Tip burn has also been observed on Swiss chard.

Sugar beet tip burn must be distinguished from injury induced by an insect, Lygus spp. Potato tip burn (3) and hopper burn (4) present an analogous situation. Tips of Lygus-affected sugar-beet leaves wilt, die, and darken, and often a good deal of the adjacent tissue turns yellow. Such symptoms accompany dark swollen lesions or open scars on the midribs and petioles resulting from the feeding punctures. Usually also there is some distortion of the affected leaves associated with the bending of the midrib

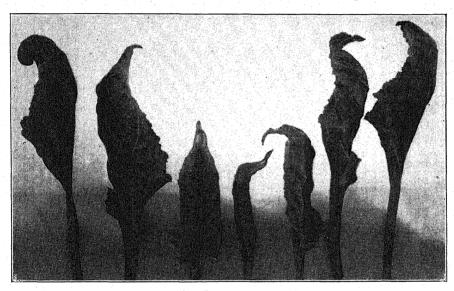


Fig. 1. Sugar-beet leaves with some of the symptoms of tip burn. Leaves distorted with blanched constricted margins and early stages of edge and tip necrosis. Leaves collected from highly fertilized field planted in September near Riverside, California. Photographed December 29, 1943.

where severely injured. Lygus injury and tip burn can be readily distinguished if one becomes familiar with both.

DISTRIBUTION

Tip burn has been observed on sugar beets in the field in California, Oregon, Washington, Idaho, Utah, Colorado, and Arizona. It probably occurs wherever sugar beets are grown, the extent or amount varying with local conditions. On mother beets in the greenhouse it has been seen or reported in Utah, Wyoming, Colorado, Minnesota, and California.

IMPORTANCE

The economic importance of sugar-beet tip burn as a disease is almost negligible. It usually occurs as merely a temporary disturbance in large, vigorous beets. It has not been observed, as is sometimes the case with lettuce tip burn, to open the way for secondary rotting organisms of importance. It does sometimes objectionably retard development of mother beets being grown in the greenhouse for breeding purposes.

EXPERIMENTAL WORK AND RESULTS

High Nitrogen Fertility

Many field observations by the junior author, beginning in 1917, on the occurrence of tip burn under conditions of high fertility led to the con-

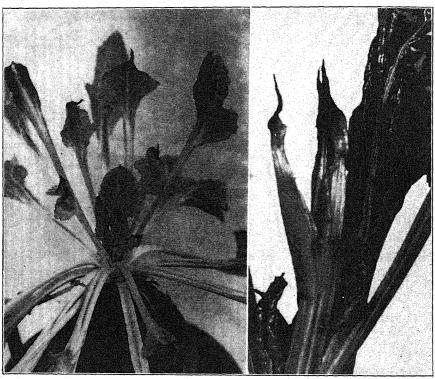


Fig. 2. Left. Tip burn of sugar beet experimentally induced by high nitrogen fertilization in the field followed by growing the plant in greenhouse under strongly reduced light. Several leaf blades are severely truncated. Large leaf at upper right shows puffy or swollen appearance of mesophyll tissue between larger veins. Photographed January 4, 1944. Right. Tip burn on end of sugar-beet seed stalk showing blackened tip of central stalk and several adjacent petioles with blackened tips but without blades. Normal young leaves on rudimentary axillary shoots are forerunners of recovery. Plant grown over winter in field at Corvallis, Oregon, and transplanted to cool greenhouse in Salt Lake City, Utah, on March 10, 1944. Photographed April 15, 1944.

clusion that the trouble is associated with very vigorous growth.² Repeated efforts were made in the field and the greenhouse to induce or in-

² Charles Price, of this same field station, observed in 1932 at Hemet, California, that tip burn was more prevalent and more severe in experimental plots that received heavy applications of nitrogenous fertilizer than in a nearby commercial field in which little or no commercial fertilizer was applied. He also observed at Chino, California, the same year, that tip burn was severe on nearly all beet plants growing in an abandoned feed yard; whereas, only a small percentage of the beets growing immediately outside this area were affected and the symptoms were relatively mild.

crease the severity of tip burn by heavy nitrogen fertilization in various ways. These gave negative or inconclusive results, though in some instances symptoms thought to be precursors of definitely diagnostic symptoms were noted. The negative results of various other types of nutritional experiments together with the fact that complete recovery consistently occurs without artificial treatment indicated that tip burn is not due to a nutrient deficiency but rather to normal constituents of the plant temporarily present in such high concentrations as to be toxic.

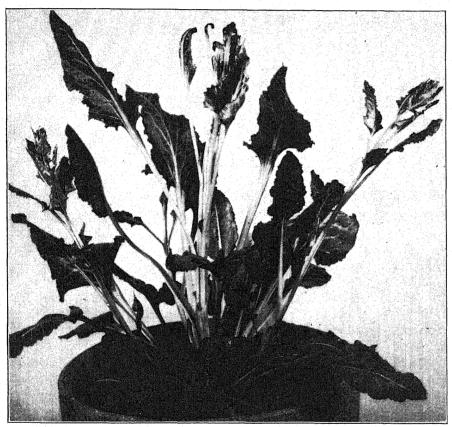


Fig. 3. Recovery from tip burn. Tip and terminal leaves of apical flowering shoot checked in growth and severely injured. Two axillary shoots at sides are developing normally. Plant overwintered in field at Corvallis, Oregon. Transplanted to cool greenhouse at Salt Lake City, Utah, March 10, 1944. Photographed April 15, 1944.

The relation of nitrogen nutrition or high soil fertility to tip burn was tested at Riverside, California, in an experiment started October 10, 1941. Thirty-six plants of the self-fertile, uniform beet variety, S.L. 68, had been obtained in fine condition. These came from the curly-top-resistance breeding field, conducted by Albert M. Murphy of this same Division, near Buhl, Idaho. The plants were from a spring planting on soil of medium fertility.

Ten of these plants were planted in ordinary potting soil and placed in

a warm greenhouse. Thirteen of the plants were set out in an outdoor bed in unmanured soil of low fertility. The other thirteen were planted in another outdoor bed with similar exposure but in fertile soil heavily manured.

On November 13, thirty-four days after the beets were set out, the plants in the greenhouse were developing normally. Growth of the plants in the two outdoor beds had been retarded by cool weather. Some of the plants in the unmanured bed showed "blackening of leaf edges suggestive of tip burn." The plants in the manured bed had more blackened tissue along the leaf edges and were slightly darker green. Apparently symptoms of tip burn developed in both outdoor lots but to a somewhat greater degree in the highly fertile soil.

On March 13, 1942, two of the thirteen plants in the low-fertility bed had tip-burn symptoms. Five of the thirteen plants in the high-fertility bed had tip-burn symptoms.

On April 19, none of the plants in the low-fertility bed had tip-burn symptoms. Two of the plants in the high-fertility bed had definite tip-burn symptoms.

The evidence indicates that when the plants were set out some of them contained some substances in adequate amounts to give rise to tip burn. The evidence also indicates further accumulation of the tip-burn-inducing substances, when the plants were grown in soil of high fertility. Recovery from tip burn was faster in the low-fertility soil.

A somewhat comparable test with two levels of soil fertility in the same two outdoor beds was conducted with the variety S.L. 68 in 1943–1944. Seed was planted in the two beds on October 30, 1943. On January 26, ten plants from each bed were transplanted to 8-inch pots and then on February 18 these potted plants were placed in the greenhouse. Some of these plants from both fertility levels were given an extra treatment with ammonium sulphate.

On March 13, 1944, slight but definite tip-burn symptoms were noted on one plant in the high-fertility bed and on March 20 two more of these plants had slight, definite symptoms. None of the plants in the low-fertility bed and none of those in the greenhouse were so affected.

This test also affords support for the idea that, with other environmental influences equal, soil fertility or nitrogen nutrition is a factor in the development of tip burn.

Light Intensity

Relatively low light intensity³ as a factor in tip burn development in heavily fertilized beets was demonstrated by the senior author in the spring

3 After the discovery of the light relationship to tip burn was made it was learned through personal communication with E. L. LeClerg, formerly Assistant Pathologist of the Division of Sugar Plant Investigations, that while stationed at the University of Minnesota in 1937 he had experimentally induced tip burn in mother beets. He accomplished this by growing the plants in a chamber with high humidity and low light intensity.

of 1943. That low light intensity is a faotor was suggested by the fact that many mother beets and stecklings planted for another purpose at Riverside, California, on February 3, 1943, had developed tip burn following prolonged cloudy weather. After the weather cleared no more tip burn developed. Another suggestive consideration was the fact that the disease had been observed in this locality more commonly in the spring, when there is a good deal of cloudiness, than in the clearer weather of summer.

The low-light-intensity hypothesis was tested in a field experiment started May 19, 1943. The beets used were in a short row of the variety U.S. 15 in a plot planted November 7, 1942, for another purpose by Charles Price of this Division. The plants had been given uniform cultural care, up to April 22, 1943, and had been fertilized with sodium nitrate at about 250 pounds per acre on January 11 and again on March 18. On April 22, a heavy application of ammonium sulphate was made to one third of the row; a similar amout of calcium nitrate was applied to a second third of the row; the remaining third of the row received no additional fertilizer. The fertilizer applications were repeated on April 26.

Four plants were defoliated in the section of row normally fertilized and four in each of the two sections heavily fertilized. Two defoliated beets of each group of four were left in full sunlight. The other defoliated plants of each group were subjected to greatly reduced light intensity by inversion of a five-gallon paper ice-cream carton over each plant. Two openings 3×5 inches had been cut in the sides of each carton near the bottom. These opening permitted some ventilation but were coverd with dark cloth to exclude most of the light.

Tip-burn symptoms began to appear on the shaded, defoliated plants after ten days. Finally, all four of the shaded, heavily fertilized plants developed tip burn. Those that had received ammonium sulphate were more severely affected than those that had received calcium nitrate. The two shaded beets that received only normal fertilization did not develop tip burn. No tip burn occurred in the exposed, defoliated plants in all three sections of the row, nor in the nondefoliated plants in this row and in other parts of the field.

Other beets from the same experimental row in the field were used in a second light-relationship experiment in the greenhouse. The plants were removed from the field on May 19, held under uniform conditions of cold storage until June 5, and then planted in pots in the greenhouse. Some of the plants were exposed to full sunlight while others were held in dense shade as in the field experiment. The plants that had been taken from the section of the row receiving only normal fertilization did not develop tip burn whether exposed or shaded. The plants from the two sections given additional fertilization did not develop tip burn if exposed to the full sunlight but did develop the disease if shaded.

The results of these two experiments prove that under conditions involved low light intensity was a controlling factor in the causation of tip

burn. The results also support and confirm the earlier evidence that nitrogen nutrition is a factor in tip-burn causation.

Additional field and greenhouse experiments were conducted to confirm the evidence that low light intensity is a factor in the development of tip burn. The results with those of the first two experiments, proved that with beets preconditioned by growing under conditions of high nitrogen fertility tip burn will develop if the beets are then grown under very low light intensity. The results of these experiments are summarized in table 1.

Plants fertilized with ammonium sulphate and exposed to full sunlight for six days and then placed in reduced light failed to develop tip burn. Those similarly fertilized and exposed to full sunlight for three weeks and then placed in reduced light developed the disease.

When plants were shaded for two months after severe symptoms of tip burn had developed, the symptoms on the leaves appearing later were pro-

TABLE 1.—Influence of low light intensity and nitrogen nutrition on sugar-beet tip burn

			Ligh	ht intensity	
Nitrogen nutrition preconditioning	Place of tests	Full st	ınlight	Reduced s	sunlight
		Plants treated	Plants diseased	Plants treated	Plants diseased
Moderate	Greenhouse Field	No. 8	No. 0	No. 8	No. 0
High		8 59 7	0 0 0	8 59 7	0 57

gressively milder until finally leaves developed showing no evidence of tip burn. This suggests that the substances involved in the development of tip burn may be present in the plant in limited amounts. It also indicates that when these substances are depleted the plants again develop normally, even though they are still in a reduced light intensity.

Reduction of the light intensity was effected in other ways in addition to the use of the paper ice-cream cartons. Unbleached muslin bags, cheese-cloth cages, and well-ventilated cardboard cartons were used. The light intensity under the unbleached muslin bags, sufficiently low to induce severe tip burn, was only 15 per cent of the full sunlight intensity on a clear day. This was determined with a pyrheliometer at the weather station of the University of California Citrus Experiment Station. The light target of the pyrheliometer was covered with an unbleached muslin bag for five minutes during a period of maximum light intensity on a clear day. Equilibrium was established within three minutes. The readings taken immediately before and after the instrument was shaded were the same. The ice-cream cartons, according to measurements with the same instrument, seemed to eliminate nearly all light, but the fact that they admitted a little light is evident because some chlorophyll developed in leaves of plants

covered by the cartons. Pyrheliometer records made by the same instrument on heavily overcast days in February and March showed that the light intensity for fully exposed plants was slightly lower for short periods of some days than under the muslin bags on bright days.

Genetic Variability

Genetic variability also appears to be a factor in the response of sugar beets to the environmental influences conducive to tip burn. It has been consistently observed in fields with uniform fertilization and general cultural care that individual plants with tip burn are distributed at random. All varieties of sugar beets now in commercial use are highly heterozygous. Further evidence of the relation of genetic composition to tip burn has been afforded by the fact that among breeding stocks and commercial varieties studied under uniform environmental conditions there will be more cases of tip burn among the more vigorous or yield-type varieties, such as U.S. 14, than among the less vigorous or sugar-type varieties, such as U.S. 15.

Alternating Light Intensities

The relation of alternation of light intensities to the development of tip burn and to recovery from the disease was tested experimentally. Plants that had been predisposed to tip burn from the standpoint of nitrogen nutrition were used. Such plants were alternately held for periods of two weeks or more in full sunlight and reduced light. (Table 2). Beets that

TABLE 2.—The relation of alternating high and low light intensities to development of and recovery from tip burn

Nitrogen fertilization		No of plants	No. of plants with tip burn after successive treatments ^a at different light intensities								
treatment	1	reated	Full	light	Reduced	Full	light	Reduced	Full	light	
Moderate		. 8		0	0		0				
Heavy		. 11		0	11		0 .			•••	
Heavy		. 5		0	5		0	5		0	

^a The plants were held continuously under a given light intensity for 14 days or longer.

had accumulated nitrogen reserves developed tip burn in low light intensity then recovered in high light intensity and then again developed tip burn if again held under low light intensity.

Food Reserves

The substances which are involved in the toxic reaction in tip burn probably accumulate with other reserves in the beet root. These substances manifest themselves through tip burn on large well-nourished beets when such plants are defoliated and shaded enough to retard or preclude photosynthesis. Under such circumstances the toxic substances would appear to come from the roots. The fact that some defoliated beets that were grown in sphagnum moss were noted by Bennett of this Division to show tip burn

on the new leaves supports the idea that the toxic substances arise directly or indirectly from storage products in the roots.

Movement of the curly-top virus in beets has been shown by Bennett (5) to be in the phloem and in the direction of flow of elaborated food. If the movement of food in a beet in reduced light is the result of mass flow of the fluid contents of the phloem from a point of high pressure (the root) to one of lower pressure (the top) any toxic substances present would move up with the sugars.

Experiments were conducted to test the hypothesis that tip burn is dependent on the direction of flow of food in the plant. Six large beet roots were potted and heavily fertilized. They were grown in full sunlight in the greenhouse until 15 or more leaves had developed. The three smallest inner leaves were then shaded with a muslin bag. The bag was so adjusted that all new leaves coming out later would be inside it. The remainder of the leaves were left on the plant and exposed to full sunlight. When 12 to 16 leaves, all healthy, had developed in the reduced light under the muslin bags, adequate time for tip burn to develop, all the outer leaves and all but six of the smallest leaves which had developed under the bag were removed from the test plants. The six smallest leaves were kept in reduced light. The plants were kept shaded until 10 to 14 additional leaves had developed under the reduced light. All plants developed tip burn. The shades were then removed and the plants exposed to full sunlight. All plants then recovered. A control plant of the same lot left continuously in full sunlight remained healthy.

A second test, in which 9 plants were used, was conducted in a similar manner. Cloudy weather prevailed for two weeks after the roots were potted and as a result all the plants had mild symptoms of tip burn on the first leaves that came out. This showed that all the plants were capable of developing tip burn. The weather then cleared and 16 to 20 new healthy leaves developed in full sunlight. The three smallest leaves of each plant were then shaded with a muslin bag. The plants were kept shaded until 10 to 14 additional leaves had developed under reduced light. The outer leaves were removed from 5 plants while the remaining 4 plants served as controls. Tip burn developed on the plants from which the outer leaves had been removed. No tip burn developed on the controls. Then the leaves of the 4 control plants were also removed. These all then developed tip burn. Thus, a total of 9 plants developed tip burn when the center leaves were shaded and the outer leaves removed.

Two experiments were conducted to demonstrate that a sufficient reduction in the light intensity for tip burn to develop was also sufficient to force the plant to draw on the reserves stored in the root. In the first test 20 beets ranging in weight from 216 to 475 grams were dug from a field plot and defoliated. The sucrose, determined on cores removed from the roots, averaged 14.8 per cent. The roots were then planted in 8-inch pots and shaded sufficiently to induce severe tip burn. The beets were left shaded



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until about 20 leaves had appeared on each beet. The shades were removed and a second core removed from each beet for sucrose determinations. Sucrose at the end of the test averaged 8.4 per cent. During the period the beets were shaded they lost an average of 43 per cent of their total sucrose. All but 6 of the 20 beets had severe symptoms of tip burn. Of the 10 largest beets (288 to 475 grams) 8 developed tip burns, whereas only 6 of the 10 beets smaller than 288 grams developed symptoms of tip burn. This evidence suggests that there may be a correlation between beet size and development of tip burn.

This experiment was repeated in the field, shading approximately 100 beets in all. Every other beet in a section of 4 rows was defoliated and a core removed from the roots with a cork borer. The beets were then shaded with a fine weave cheesecloth. This cloth when clean reduced the light intensity 68 per cent when placed over a pyrheliometer on a clear day. After 12 to 16 leaves had developed on the defoliated plants the shades were removed and cores removed from both the defoliated roots which were sampled at the beginning and also from the undefoliated roots.

In this test the reduction in the amount of light by shading was sufficient to cause only a 10 per cent loss in sucrose from the roots of the undefoliated plants and a 26 per cent loss in the sugar content of the defoliated plants. Mild though distinct symptoms of tip burn developed on approximately 25 per cent of the shaded plants. Mild cupping of the leaves, an incipient stage of tip burn, was observed on approximately 25 per cent more of the shaded plants. The weights of these roots were not known, but it is certain that many roots were small. The size relationship in conjunction with the higher light intensity used may explain the low percentage of the plants which developed tip burn. No tip burn was observed in the remainder of the plot during the experiment.

DISCUSSION

The fact that tip burn develops in the field during prolonged periods of cloudy or foggy weather suggests the need to consider humidity as a possible factor. The possibility of a temperature relationship should also be considered. Relative humidity, at least as it prevailed in the greenhouse, appeared not to influence the development of tip burn in the test plants used. In view of the wide range of temperature under which tip burn has been observed to develop, there appears to be no critical range of temperature required.

The substances that cause sugar-beet tip burn are probably nitrogenous compounds but since the injury does not appear immediately after plants are heavily fertilized and shaded the effect is likely not due to the ammonium or the nitrate ion. The injury is probably due to complex nitrogenous compounds intermediate between the ammonium ion and the proteins.

If, as is apparently the case, the toxic substances accumulate in the roots and if they move up with the food in the phloem, a hypothetical ex-

planation can be advanced as to why tip burn did not develop on plants with the younger, inner leaves in reduced light and the outer leaves in full sunlight. In such cases perhaps the exposed outer leaves would supply the food requirements of the covered leaves so that there would be little or no movement of substances from the root to the covered leaves through the phloem.

Another hypothetical explanation of the fact tip burn did not develop on the covered inner leaves when the outer leaves were exposed to full sunlight is that perhaps products of photosynthesis combine with the toxic substances and in effect neutralize them. Under such conditions perhaps the substances that cause tip burn would be reduced to nontoxic concentrations.

If the tip burn is due to translocation of substances in the phloem from the root to the tops under reduced light then it would follow that recovery from tip burn under full sunlight would be due to a reversal of the direction of the flow of the phloem contents. If, on the other hand, tip burn is due to substances not adequately neutralized by products of photosynthesis under reduced light, then one could postulate that the increased amount of photosynthates under high light intensity would hold the toxic substances below the critical concentration.

SUMMARY

Tip burn of sugar beet is a disease believed due to toxic concentrations of substances normally present in the plant.

Tip burn develops when beets that have been grown in fertile soil with an abundance of nitrogen for a relatively long time are then grown under a low light intensity. The nitrogen and the light factors are both necessary.

When the outer leaves are left on the beet plant and in full sunlight tip burn does not develop on the new leaves even though they are shaded.

The toxic substances involved in tip burn are apparently accumulated in the root and are probably normal nitrogenous constituents of the plant temporarily in excessive concentrations.

Complete recovery from tip burn occurs if affected beets are grown under high light intensity and conditions otherwise favorable for beet growth.

Sugar beets are, in general, highly heterozygous and vary widely in their sensitiveness to the complex of factors causing tip burn.

U. S. DEPARTMENT OF AGRICULTURE, SUGAR PLANT FIELD LABORATORY, RIVERSIDE, CALIFORNIA.

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PATHOGENICITY OF THE VASCULAR FUSARIUM OF GLADIO-LUS TO SOME ADDITIONAL IRIDACEOUS PLANTS

W. D. MCCLELLAN1

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Members of the Iris family are frequently grown in association by both commercial and amateur growers. With Fusarium rapidly increasing in importance in Gladiolus production the question arose as to whether or not plants of related genera in the Iridaceae would become infected with the vascular Fusarium from Gladiolus recently described by McCulloch (4) as Fusarium orthoceras App. and Wr. var. gladioli. Since 1941 plants of 11 genera in the Iridaceae have been inoculated with isolates Nos. 12–1, 16–1, and 19–1 from Miss McCulloch's collection, with positive infection resulting in plants of 9 genera and possible infection in plants of 2 genera.

MATERIALS AND METHODS

The plant materials tested were Homeria collina Vent., Neomarica gracilis Herb., Streptanthera cuprea Sweet, Tritonia crocata Ker., T. lineata (Salisb.) Ker., Babiana hybrids, Crocus (spring-flowering, mixed), Ixia hybrids ("Bloem Erf," "Dutch," and "Mrs. Cleveland's"), Sparaxis (assorted), and Watsonia (assorted), all from commercial sources. Bulbous iris varieties Imperator, Poggenbeek, and Wedgewood were from stock grown at the Plant Industry Station, Beltsville, Md., and corms of Freesia were from seedlings grown in sterilized soil at the Station. Corms of Tritonia crocata used in 1944 were also seedlings grown at Beltsville.

In the 1941–42 tests all of the bulbs and corms were grown in sterilized soil in 5-inch pots and, with the exception of the bulbous *Iris* and the *Watsonia*, were planted 3 to a pot. In the 1942–43 and 1944 tests sterilized sand was used and the plants were fed weekly with a nutrient solution. In these tests the plants, with the exception of bulbous *Iris*, were grown in 4-inch pots. The bulbous *Iris* was grown in flats of sterilized sand. The *Iris* was planted 24 per flat, the *Watsonia* and *Crocus* 1 per pot, and other genera 3 per pot.

Some of the corms and bulbs were infected with *Fusarium* when they were planted, but the likelihood of contamination was kept at a minimum by the placing of all pots in sterilized saucers, by a wide spacing of the pots, and by care in watering. In spite of these precautions some contamination occurred, chiefly in 1941–42 when the space used was greatly reduced to provide room for war emergency crops. While the plants used in these experiments were being moved into smaller space the pots were allowed to come in contact with each other.

¹ Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland.

The 1941–42 inoculum was prepared from isolates 12–1, 16–1, and 19–1 grown on sterile oats for 2 weeks. Inocula of the 3 isolates were mixed and about 40 cc. of the mixture placed over some soil in the bottom of each pot. The inoculum was covered with soil, the bulbs or corms were then planted, and the pot was filled with soil. An equal amount of sterile oats was placed in each uninoculated pot.

TABLE 1.—Infection of representatives of 11 genera of the Iridaceae with the vascular Fusarium from Gladiolus

			in- lated		In- ulated	
Plants tested	Year	No. of bulbs, corms,	Fusarium isolateda	No. of bulbs, corms,	Fusarium isolateda	Type of infection in bulbs, corms, rhizomes
Babiana, hybrids Crocus (mixed, spring-flowering) Freesia, seedlings Homeria collina Vent. Iris (bulbous) var. Imperator var. Poggenbeek var. Wedgewood Ixia, hybrids, "Bloem Erf" "Dutch" "Mrs. Cleveland's" Neomarica gracilis Herb. Sparaxis, assorted Streptanthera cuprea Sweet Tritonia crocata Ker., seedlings	1941-42 1942-43 1944-42 1941-42 1942-43 1942-43 1941-42 1942-43 1941-42 1942-43 1941-42 1942-43 1941-42 1942-43 1941-42 1941-42 1942-43 1941-42	6 6 6 15 18 9 3 4 4 24 4 24 3 3 3 3 3 3 3 3 4 4 24 4 24 5 3 5 6 3 3 4	0/6 0/6 1/3 1/17 0/9 0/1 1/3 1/2 0/4 7/23 2/2 0/3 1/1 0/3 1/2 0/1 0/2 0/6 ? 0/2 0/2	9 18 18 45 40 15 9 14 48 48 12 48 9 9 9 9 9 9 18 9 18	4/5 3/18 13/18 7/23 33/43 3/15 1/5 1/12 9/34 13/21 7/9 18/29 6/7 2/9 6/6 6/6 6/9 1/2 6/9 12/18 5/7 9/9 1/3	Vascular do do and basal Vascular do do do and basal Basal do do do do do do do do No symptoms Vascular Basal Slight vascular in base do Vascular
T. lineata (Salisb.) Ker. Watsonia, assorted	1944 1942–43 1941–42 1942–43	9 3 4 10	0/9 0/3 0/4 0/5	15 9 16 29	3/15 0/6 6/16 4/9	do No symptoms Vascular and basal do

^a The numerator indicates the number of successful isolations; the denominator, the number of bulbs from which isolations were made.

For the 1942-43 and 1944 tests each of the three isolates was grown in a modified Richard's solution and ground in a Waring Blendor. The bulbs and corms were dipped just before planting in a mixture of the three isolates.

Isolations were made from many of the corms or bulbs when recognizable foliage symptoms appeared. In many instances the corms or bulbs had

rotted so badly that isolations were not attempted. Isolations were made from most of the remaining bulbs at maturity.

Representative isolates recovered from inoculated plants of each susceptible genus except *Crocus*, *Homeria*, *Neomarica*, and *Tritonia* have been tested for pathogenicity in *Gladiolus*. Twelve corms each of the *Gladiolus* varieties Picardy and Dr. F. E. Bennett were dipped in a suspension of a mixture of representative isolates from a given genus and planted in sterilized sand in 8-inch pots, 3 corms to a pot. These corms were fed weekly with a nutrient solution.

RESULTS

The results of the inoculations are in table 1. Since isolation was usually attempted from the vascular system or the base of the corm or bulb but seldom from the roots, Fusarium was not always recovered from plants which were obviously diseased. Positive infection was obtained in the following: Babiana, Crocus, Freesia, bulbous Iris, Ixia, Sparaxis, Streptanthera, Tritonia, and Watsonia. Typical reddish-brown vascular discoloration occurred in one or more bulbs or corms in each of these. In Neomarica, however, no recognizable symptoms were present in roots, tops, or rhizomes. Whether true infection existed in Neomarica is open to question. Rotting of the roots and corm or bulb base was a general symptom of the effects of this Fusarium in all genera. This effect was the predominating symptom complex in the bulbous Iris whereas the characteristic symptom in Crocus, Ixia, and Watsonia was the typical reddish-brown vascular discoloration. Detailed symptoms are described.

Babiana.—The thin leaves became yellow and died back from the tips leaving the dead, yellow-brown tissue thin and paper-like. This necrosis gradually proceeded down the leaf towards the corm. Plants remained alive in the greenhouse as long as 5 months after inoculation. The central vascular cylinder was discolored if infection had progressed sufficiently. Frequently this brown discoloration was confined to the base of the vascular cylinder but in one plant it extended throughout the central cylinder and into the stem. In this plant the parent corm appeared to be normal, the fungus entering the daughter corm through the contractile roots.

Crocus.—Foliage symptoms first appeared within 4 weeks after inoculation on one of the plants and were present within 9 weeks in all but
one of those inoculated. The first symptom, a slight yellowing at the tips
of the leaves, was soon followed by necrosis as the yellowing progressed
down the leaves. Many of the leaves were twisted or curled (Fig. 1) and
in 1 plant this curling was extremely severe. Stunting also occurred.
One plant grew very little and was nearly dead 5 weeks after inoculation.
The roots usually rotted off; thus the absence of roots (Fig. 1) and the
presence of basal corm rot were distinguishing symptoms. This light
brown basal rot sometimes involved the lower half of the corm, and brown
node lesions were frequent in the corms. Vascular discoloration was absent

in most of the corms, but in 2 of these the discoloration extended through the parent corm into the daughter corm (Fig. 1).

The symptoms produced in *Crocus* by the Gladiolus *Fusarium* were very similar to those illustrated in *Crocus* by Drayton (3) and by Moore (5). It is probable that these workers were dealing with the same *Fusarium* as the writer. Drayton reported that Crocus rot was of serious consequence in Holland and that it is primarily a basal decay which advances further during storage and may completely rot the corm. Moore said that the Fusarium rot was the most prevalent and destructive disease of Crocus corms; and Pape (6) mentioned a Fusarium corm rot which frequently

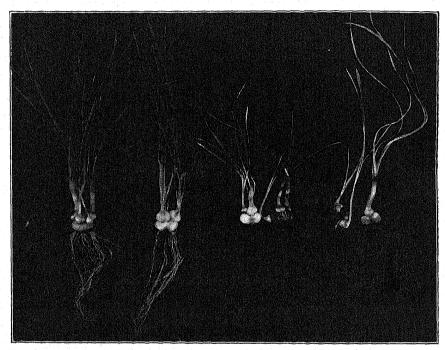


Fig. 1. Crocus plants grown for 60 days in sterilized sand, fed weekly with a nutrient solution. The two plants at the left were not inoculated. Those at the right were inoculated by dipping the corms in a spore and mycelium suspension of the Gladiolus Fusarium.

completely destroyed the corms in storage. Attempts at infection of Gladiolus with the Fusarium obtained from Crocus were not reported by these writers nor was the possible relationship between the Gladiolus Fusarium and the Crocus Fusarium suggested. Abe (1) described a root and corm rot of Crocus sativus L. which is said to be caused by Fusarium bulbigenum Cke. et Mass. var. blasticola (Rostr.) Wr. According to the system of Snyder and Hansen (8) this would become F. oxysporum Schl. Abe was unable to infect onion seedlings with this Fusarium.

Freesia.—Freesias used in these tests have been very susceptible to the Gladiolus Fusarium. Yellowing of the foliage was evident within 30 days

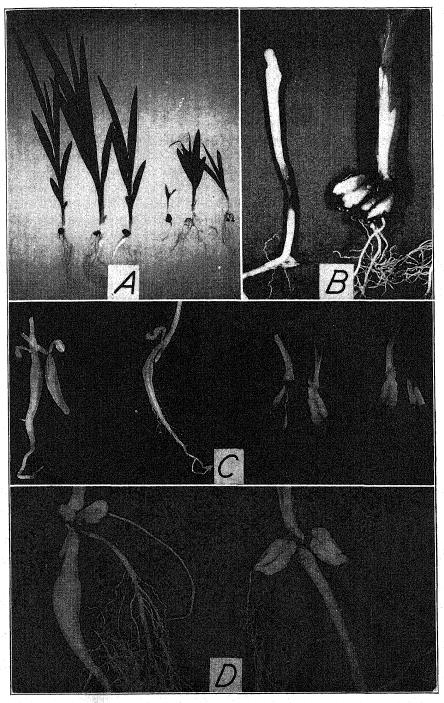


Fig. 2. Freesia plants grown in sterile sand, fed weekly with a nutrient solution. A. Right, plants 63 days after corms were inoculated by dipping them in a spore and mycelium suspension; left, not inoculated. B. Lesion on the main root (left); core rot of parent corm (right). C. Sections through healthy corms (left); diseased corms (right) showing basal and core rot. Plants are of the same age. D. Plants of the same age showing retardation of corm development following inoculation (right), compared with normal development (left). Note that the parent corm of the healthy plant has almost completely shriveled and that the large fleshy root is swollen.

after inoculation, and within 60 days many of the tops were completely dead and the corms rotted. The most pronounced effect of this Fusarium on Freesia was the stunting and retarding of the development of the entire plant. Even though no foliage yellowing was evident, growth was but one-third to one-half that of uninoculated plants (Fig. 2, A). The shriveling of the parent corm and the swelling of the main root was greatly retarded in inoculated plants (Fig. 2, D). The first symptom, in addition to stunting, was a yellowing and dying back of the foliage. Frequently the leaf tips were white rather than yellow, similar to a physiological burn. This die-back was accompanied by a decay of the root tips, by root lesions (Fig. 2, B), by vascular discoloration, and finally by complete rotting of the corms (Fig. 2, B and C). The infected central vascular core was either brilliant red or dark brown, this discoloration frequently extending through both the parent and daughter corms into the leaf bases. Occasionally, also, the radiating vascular strands were discolored. As the rot progressed the base of the corm rotted and surface lesions appeared elsewhere on the corm.

Taubenhaus and Ezekiel (9), reporting a Fusarium wilt and corm rot of Freesia, described and figured symptoms similar to the above but they did not mention a retardation in the development of the daughter corm. They reported only slight decay of the basal plate and very little stunting when Freesia corms were inoculated with a Fusarium from decayed Gladiolus corms, whereas isolates of Fusarium from Freesia, tomato, and cabbage were reported as causing a typical core rot. Corms of Gladiolus that were planted in soil infested with the Freesia Fusarium showed a slight decay at the bases which prevented the formation of new roots. As McCulloch (4) has pointed out, their failure to obtain infection may have been due to the use of varieties which were resistant to the strain of Fusarium used in the tests. Similarly McCulloch (4) was unsuccessful with cross inoculations with Fusaria from Gladiolus and from Freesia, and she believed her failure may have been due to the use of resistant varieties as test plants. She stated that the Fusariuminfected Freesia corms had symptoms similar to the vascular disease of Gladiolus and that the Fusarium isolated from these corms appeared to be identical with her Gladiolus Fusarium.

Homeria collina.—The symptom complex has not been well established for this species. As observed, the leaves died back from the tips and, when plants were lifted for examination, the corms looked healthy but all the basal roots were either dying back or had rotted away. Many of the contractile roots were brown at the tips. Only slight vascular discoloration was seen in the bases of 2 corms nearly a year after inoculation.

Iris (bulbous).—One or more species of Penicillium appear to be closely associated with Fusarium infection in bulbous Iris. Many Iris bulbs first inoculated and infected with Fusarium soon had Penicillium fruiting freely between the rotting bulb scales. No doubt Fusarium rot of bulbous Iris is frequently confused with the Penicillium rot described by Moore (5). Fusarium infection was characterized by a stunting of the plant and by the darkened bulb bases and the absence of roots (Fig. 3). If secondary in-

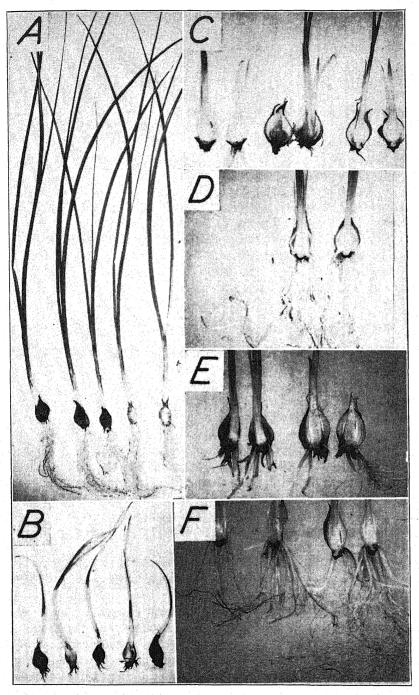


FIG. 3. Effect of the Gladiolus Fusarium on bulbous Iris. All plants are the same age. A-B. Wedgewood not inoculated (A), inoculated (B). Note the difference in growth and the absence of roots. C-F. Longitudinal sections through diseased and healthy Imperator (C and D), and Poggenbeek (E and F), showing the discolored bases and bulb scales in the inoculated bulbs (C and E).

fection by Penicillium did not occur the bulb remained firm with a badly discolored and rotted base. Browned vascular tissues frequently were noted in the bulb scales and occasionally vascular discoloration extended into the stem, but usually the discoloration was confined to the base of the bulb (Fig. 3). Fusarium was readily isolated from such areas. Roots were few and poorly developed or had been rotted off altogether (Fig. 3). No differences in susceptibility of the 3 varieties tested were noted although F. P. Me-Whorter, in a personal communication, stated that the Poggenbeek variety is severely affected by a Fusarium basal rot in the field, and Creager (2) stated that a Fusarium is very destructive to the Cajanus, Hart Nibbrig. and Imperator varieties. A mixture of bulbs of Wedgewood and other varieties was sent the writer from Grants Pass, Oregon, by McWhorter. These bulbs had darkened bulb bases typical of those inoculated with the Gladiolus Fusarium. A Fusarium similar to the Gladiolus Fusarium was isolated from both the Wedgewood and David Haring varieties of Iris, but no infection was obtained in either the Picardy or the Dr. F. E. Bennett variety of Gladiolus with either isolate. A Fusarium isolated from bulbous Iris from Texas likewise produced no infection in these 2 varieties of Gladiolus. This discrepancy in behavior between the Gladiolus and the Iris isolates is as yet unexplained but may be due to differences in behavior between strains of Fusarium. Strains of the Gladiolus Fusarium differing in pathogenicity are known to exist (unpublished data).

Creager (2) has published a note on a root and bulb rot of both Spanish and Dutch *Iris* which is caused by a *Fusarium* "closely resembling *F. oxysporum.*" He stated that the pathogen enters the root and finally involves the basal plate and scales of the bulb. No mention was made of any cross-inoculation studies.

Ixia.—The first symptom of Fusarium infection was a yellowing of the tips of the leaves. As this yellowing progressed down the leaves these began to twist and their tips died. The roots of such plants were usually badly decayed and the vascular system of the contractile roots sometimes was discolored. In later stages all or nearly all of the roots were rotted. The base of the corm was frequently rotted and surface lesions were present. Basal rotting gradually extended upward into the central vascular cylinder, thus producing a core rot. The radiating vascular tissues were likewise often discolored. As the disease progressed it sometimes became a general corm rot and often a corky dry rot followed the discoloration of the radiating vascular tissues. Brilliant red areas frequently defined the corm lesions, but apparently the red color is not always associated with the disease since it also occurs as a result of other injuries. The daughter corm usually became infected through the connecting vascular tissue, but occasionally the daughter corm became infected from contractile roots before the parent corm was invaded.

Neomarica gracilis.—No recognizable symptoms were observed, but Fusarium was obtained from one of two plants inoculated. It is questionable whether there was true infection.

Sparaxis.—Typical vascular and core rot was less common in Sparaxis than in Freesia, Crocus, or Ixia. The leaves yellowed and died back as in these other genera, and the roots were few, poorly developed and dying back, but often there was no vascular discoloration in either the fibrous or the contractile roots. Several corms, however, had definite vascular discoloration and core rot. The core of one parent corm was rotted and this decay extended slightly into the daughter corm. Fusarium was also isolated from a discolored area in the basal plate of another daughter corm.

Streptanthera.—The leaves became yellow and died at the tips, and the plants were badly stunted. The roots rotted off and the vascular core was discolored. The discoloration was yellow brown at first, but became much darker brown as the disease progressed. Some surface rot occurred at the base of the corm and eventually both parent and daughter corms rotted completely. Fusarium was isolated from such rotting tissue as well as from the lightly discolored and the darker vascular system.

Tritonia.—In the 1941–42 tests 12 of the 18 corms of T. crocata which were inoculated and planted rotted completely and none of them sent up new shoots, whereas all of the 4 uninoculated corms grew well and were in good condition when dug 11 months later. Of the 15 seedling corms of T. crocata planted in 1944, 3 had typical vascular discoloration and the others had none. Fusarium was isolated from these 3 corms. The foliage of all of the inoculated plants had begun to die back within 30 to 45 days after inoculation whereas the uninoculated plants retained a healthy appearance. Possibly distinct varietal resistance to Fusarium such as is known to occur in Gladiolus (4) also occurs in Tritonia. Plants of Tritonia lineata inoculated in 1942–43 remained healthy.

Watsonia.—The foliage of infected plants had a burned appearance around the edges and tips and then became yellow toward the base. Most of the roots rotted off although occasional healthy roots remained. The roots might or might not be entirely rotted away, with no evidence of corm rot other than slight rotting at the root bases. Usually, however, rotting of the roots was accompanied by a basal rot of the corm and occasionally the entire corm rotted. The vascular system of 1 corm was discolored throughout and extended into the stem.

REINFECTION OF GLADIOLUS

Successful reinfection of the very susceptible variety of Gladiolus, Dr. F. E. Bennett, was obtained with the Fusaria isolated from plants of the following genera: Babiana, Freesia, Iris, Ixia, Sparaxis, and Watsonia. Only slight vascular or basal infection of the Picardy variety was obtained with isolates from these genera. Slight vascular infection of Dr. F. E. Bennett, but not of Picardy, was obtained in 3 trials involving 42 corms of each variety with isolates from Streptanthera. Reinfection of Gladiolus, has not yet been attempted with the isolates from Crocus, Homeria, Neomarica, or Tritonia.

DISCUSSION

The host range of the vascular Fusarium of Gladiolus is not confined to a single genus, but as yet these studies have not progressed far enough to determine whether Fusaria isolated from other members of the Iridaceae are pathogenic to Gladiolus. In limited trials isolates of Fusarium obtained from bulbous Iris were not pathogenic to Gladiolus. Recent investigations (7) have shown that some of the vascular Fusaria do not have the narrow host range attributed to them. This does not seem particularly surprising in view of the fact that Wellman and Blaisdell (10) were able to select some variants of Fusarium bulbigenum var. lycopersici (Brushi) Wr. and R. that were more pathogenic to tomatoes and some which were less. It would seem likely that it might be possible to select variants that would be pathogenic on closely related or even remotely related plants especially if we accept Snyder and Hansen's (8) concept of F. oxysporum Schl. and their statement that "the Fusaria which cause vascular wilts are merely biologic forms of one and the same species."

In view of the results obtained with the Gladiolus Fusarium, members of the Iridaceae should not be grown in association where the vascular Fusarium is known to be present.

SUMMARY

The vascular Fusarium of Gladiolus described by McCulloch proved to be pathogenic to plants of the following genera of the Iridaceae: Babiana, Crocus, Freesia, Iris (bulbous), Ixia, Sparaxis, Streptanthera, Tritonia, and Watsonia. Infection was uncertain in Neomarica and Homeria.

A Fusarium isolated from bulbous Iris was nonpathogenic to the Picardy and Dr. F. E. Bennett varieties of Gladiolus.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES, PLANT INDUSTRY STATION,

BELTSVILLE, MARYLAND.

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PHYTOPATHOLOGICAL NOTES

A Cross between Lycopersicon esculentum and Disease-Resistant L. peruvianum.—A group of varieties of Lycopersicon peruvianum (L.) Mill. and related species imported from Peru, South America, by the Division of Plant Exploration and Introduction, have had a most consistent resistance to tomato foliage diseases. Various workers report that lines have also been found with varying degrees of resistance to curly top, fusarium wilt, nematode rootknot, bacterial spot, and mosaic. Because of this marked resistance of certain varieties of L. peruvianum to several tomato diseases, breeders have endeavored to hybridize them with varieties of L. esculentum Mill. in order to incorporate their resistance into new superior horticultural types.

Hybrids have already been reported between Lycopersicon esculentum and L. chilense Dun.² (L. peruvianum var. dentatum (Dun.)³), between L. esculentum and L. peruvianum var. humifusum C. H. Mull., and between L. esculentum and various species of the subgenus Eriopersicon C. H. Mull. group.3,5 However, so far as the writers have been able to ascertain, no cross between L. esculentum and the type species of Eriopersicon, L. peruvianum, has heretofore been reported.

When the work here recorded was started selections were made from introductions of Lycopersicon peruvianum grown in the field tests at Belts-The plants that retained the healthiest foliage throughout the growing season were chosen as parent stocks. The pollen parent (Fig. 1, C, F. I) of the hybrid here reported is one of the more fruitful lines and bears large numbers of small greenish fruits usually tinted with purple when mature and averaging about 5 inch in diameter.

After examining several hundred seedless tomato fruits that developed in the greenhouse at Beltsville, Md., from cross pollination of numerous tomato varieties with pollen of Lycopersicon peruvianum, viable seed was obtained in a cross with Prince Borghese, an Italian paste type. This variety of L. esculentum is characterized by the production of many small flowers on large forking cymes which bear large clusters of elongated, bright red, 2-celled fruits (Fig. 1, A, D, G).

The F₁ plants were vigorous vegetative growers and developed vines about twice the size of either parental stock. These plants flowered and produced pollen in the greenhouse but set no fruit unless a growth-regulating substance such as naphthalene acetamide or indolebutyric acid was

¹ Alexander, L. J., R. E. Lincoln, and V. Wright. A survey of the genus Lycopersicon for resistance to the important tomato diseases occurring in Ohio and Indiana. U. S. Dept. Agr., Plt. Dis. Reporter Suppl. 136. 1942.

² Holmes, F. O. The Chilean tomato, *Lycopersicon chilense*, as a possible source of disease resistance. Phytopath. 29: 215–216. 1939.

³ Muller, C. H. A revision of the genus *Lycopersicon*. U. S. Dept. Agr. Miscellaneous

Publication 382. 1940.

⁴ Wright, V., and R. E. Lincoln. Resistance to defoliation disease in tomato. Purdue Univ., Agr. Exp. Stat., Ann. Rep. 53 (1940): 42-43. 1940.

⁵ Blood, H. L. Curly top, the most serious menace to tomato production in Utah. Utah Agr. Exp. Stat. Farm and Home Science 3 (1): 8-11. 1942.

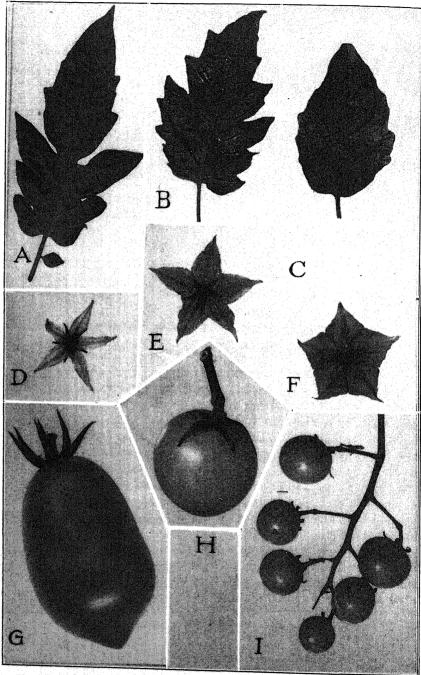


Fig. 1. Typical leaflets, flowers, and fruits of the F_1 hybrid, Lycopersicon esculentum $Q \times L$. peruvianum 3, and of its parents. Natural size. A, B, C. Leaflets of pistillate parent, of F_1 hybrid, and of staminate parent, respectively. D, E, F. Flowers of pistillate parent, of F_1 hybrid, and of staminate parent, respectively. G, H, I. Fruits of pistillate parent, of F_1 hybrid, and of staminate parent, respectively.

applied to the pedicels or peduncle when the flowers were pollinated (Fig. 1, B. E. H). However, all such fruits have been seedless. Plants propagated by cuttings when grown in an open field where many other tomato lines were growing, set fruit sparingly and very few fruits contained seed. These fruits ranged from \(\frac{3}{4}\) to \(\frac{1}{8}\) inches in diameter, and were uniformly globular, smooth, yellow, and usually 2-celled. A small population of F, plants has been grown from seeds obtained from the field-grown open-pollinated F₁ plants. These plants had marked phenotypic differences and most of them have been barren. Several outcrosses have been made to Pan America, Rutgers, and various hybrid combinations of L. esculentum. The progenies of all these outcrosses show wide segregations in plant habit, in foliage and fruit characters, and in fruitfulness. About 25 red-fruited and vellow-fruited selections have been made from these outcrosses. The selections are fruitful, the fruit ranging in diameter from $1\frac{1}{2}$ to $2\frac{1}{2}$ inches. Since these progenies were derived from open-pollinated field selections their genetic composition is somewhat uncertain because of the probability of some chance field crossing. However, this should not hinder the development of disease-resistant horticultural varieties from these interspecific outcrosses provided the resistance factors can be retained while breeding for fruit size, quality, and productiveness.—W. S. Porte, Pathologist, and H. B. Walker, Scientific Aid, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture.

Varietal Resistance of Tomato Seedlings to the Stem-Lesion Phase of Alternaria solani.¹—One of the most serious disease problems connected with the production of tomato seedlings in the South for use in the northern canning areas is stem infection by Alternaria solani.² Considerable data³.⁴ have been compiled on methods which aid materially in reducing infection under field conditions but nothing has so far approximated complete control. In view of the many difficulties involved in control by cultural practices and spraying, resistant varieties appear to offer the best solution of this problem. Reynard and Andrus⁵ showed that certain strains of tomato have considerable resistance to both the collar rot and the stem-canker caused by A. solani, and they suggested the possibility of breeding this resistance into canning varieties of tomatoes now being grown by northern farmers. In order to

¹ A phase of cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture; the Georgia Coastal Plain Experiment Station; the Georgia Agricultural Experiment Station; and the Georgia State Department of Entomology.

² Moore, W. D., H. Rex Thomas, and Edward K. Vaughn. Tomato seed treatment in relation to control of *Alternaria solani*. Phytopath. 33: 797-805. 1943.

Moore, W. D. Some factors affecting the infection of tomato seedlings by Alternaria solani. Phytopath. 32: 399-403. 1942.
 Moore, W. D., and H. Rex Thomas. Some cultural practices that influence the de-

⁴ Moore, W. D., and H. Rex Thomas. Some cultural practices that influence the development of Alternaria solani on tomato seedlings. Phytopath. 33: 1176-1184. 1943.

⁵ Reynard, George B., and C. F. Andrus. Inheritance of resistance to the collar-rot phase of Alternaria solani on tomato. Phytopath. 35: 25-36. 1945.

study some of the resistant strains under commercial conditions, tests with a number of selections from the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C., were started in 1942 at Tifton, Ga., by the senior author and continued through 1944.

Seed beds were prepared during March of each year by turning the soil, harrowing, and broadcasting a 4–8–4 fertilizer at the rate of 1,000 pounds per acre. The soil was smoothed with a drag and the tomato seed planted approximately an inch deep by hand in rows 16 inches apart and at the rate of 20 seed per foot. The beds were watered as needed with an overhead sprinkling system and the rows cultivated with a hand cultivator to keep down grass and weeds. About mid-May, when the seedlings were approximately 8 inches tall, 25-plant lots were pulled at random from each plot. The roots and lower portion of the stems were wrapped with wet moss and paper and the plants were then stored at room temperature for 48 hours. Following storage, 4 replicates of 25 plants from each strain (15 plants were used in 1944) were set twelve inches apart in rows 3 feet apart. The plants were allowed to grow for periods ranging from 21 to 36 days, after which they were carefully pulled from the soil, thoroughly washed, and the number of stem lesions recorded.

The 1942 test consisted of 13 tomato strains as follows: one each of Marglobe, Indiana Baltimore, King George, Danish Early, Targinnie Red, and Devon Surprise; two selections of Norduke, two of Marglobe × Red Currant cross, and three of Riverside. The number of stem lesions varied from a mean of 158.5 per replicate on Marglobe to 4.5 on King George. There were five strains with means varying from 4.5 to 8.2, four with means from 10.2 to 22.0, and four with means from 64.7 to 158.5.

As a result of growth studies made during the summer of 1942 several of the strains were dropped and others were added to give the following selections for the 1943 test: three from Targinnie Red and one each from a Red Currant × Marglobe cross, a Devon Surprise × Marglobe cross, a Targinnie Red × Montgomery cross, Devon Surprise, Norduke, and Marglobe. The mean number of stem lesions per replicate in this test varied from 213.7 on Marglobe to 0.2 on Norduke. Four strains had lesions varying from 0.2 to 1.0, four had from 5.5 to 24.0 lesions, and one had 213.7 lesions.

Additional eliminations were made during the summer of 1943 and the 1944 test included only 5 selections. They were as follows: three F_3 selections from the original Marglobe × Devon Surprise cross outcrossed to Pan America, one F_4 selection from a Cooper Special × Devon Surprise cross, and one Marglobe. The mean number of stem lesions in this group varied from 248.2 on Marglobe to 25.5 on the Cooper Special × Devon Surprise cross. The three selections from the Marglobe × Devon Surprise × Pan America strain had lesions varying from 34.0 to 38.0.

While these strains do not have complete resistance to stem infection by Alternaria solani and are not comparable to the average canning tomatoes in size and quality of fruit, they possess definite possibilities as stock that

may be used for crossing with other varieties in a disease-resistance breeding program. The pure lines of Targinnie Red, Devon Surprise, Norduke, and their several crosses were significantly more resistant to stem infection by Alternaria solani than Marglobe in all tests during the three seasons in which this study was made.—W. D. Moore, Pathologist, and George B. Reynard, formerly Assistant Geneticist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

Meadow Nematodes as the Cause of Root Destruction.—The year 1889 is notable in the history of research on plant-parasitic nematodes in the United States as the year of publication of the first papers on this subject matter in this country, namely Atkinson's bulletin on the root-knot nematode1 and also that of Neal.² There is, however, a third publication of the same year which is generally overlooked: F. Lamson Scribner's "Diseases of the Irish Potato," an important part of which deals with nematode diseases. Scribner also reports root knot, but in addition a second nematode affliction of potatoes is described. The causative nematode is not named but it is easily recognized by description, figures, and symptoms as a meadow nematode, which has been named Scribner's meadow nematode.4

While the root-knot nematode has received considerable attention as a disease factor, the meadow nematodes—plant-parasitic nematodes of the genus Pratylenchus Filipjev—have been almost completely ignored. This is unfortunate. The pratylenchs are doubtless one of the most important primary factors in root destruction among cultivated and uncultivated plants. The overall damage caused by these pests in the United States is possibly greater than that which is attributable to the root-knot nematode. There are several obvious reasons for this underestimation. Meadow nematodes do not produce specific symptoms. The lesions they cause on roots are not different from those produced by many other nematodes or from those induced by many fungi and bacteria. Excised root sections placed on culture media will not bring them to light since they will perish under these abnormal conditions. Meadow nematodes are migratory, often almost completely evacuate a decaying root, and may not be found at all even if the root is carefully examined. They are very small, mostly 0.350 to 0.800 mm. in total length. They may enter a root at any place, even in very woody portions. However, they appear not to enter the vascular system but to

Atkinson, George F. A preliminary report on the life history and metamorphoses of a root-gall nematode, Heterodera radicicola (Greeff) Müll., and the injuries caused by it upon the roots of various plants. Science Contrib., Alabama Agricultural Experiment Station 1: 177–226. 1889. Also published as Ala. Agr. Exp. Stat. Bull. 9. 1889.

2 Neal, J. C. The root-knot disease of the peach, orange, and other plants in Florida,

due to the work of Anguillula. U. S. Dept. Agr., Div. Entom. Bull. 20. 1889.

³ Scribner, F. L. Diseases of the Irish potato. Tenn. Agr. Exp. Stat. Bull. 2(2):

⁴ Sherbakoff, C. D., and W. W. Stanley. The more important diseases and insect pests of crops in Tennessee. Tenn. Agr. Exp. Stat. Bull. 186. 1943.

migrate mainly in the cortical tissues, rarely in the pith and its rays. Other subterraneous formations of a plant (bulbs, corms, tubers, rhizomes, in rare instances even basal portions of stems) appear subject to attack. Eggs may be deposited in the tissues of a host plant or in the soil. Not infrequently aggregations of specimens form nests of infection which may ring a root and thus amputate its distal portions. It is obvious that even a small number of specimens may, by this mode of attack, greatly interfere with the functioning and growth of a root system. The plant often attempts to repair the damage

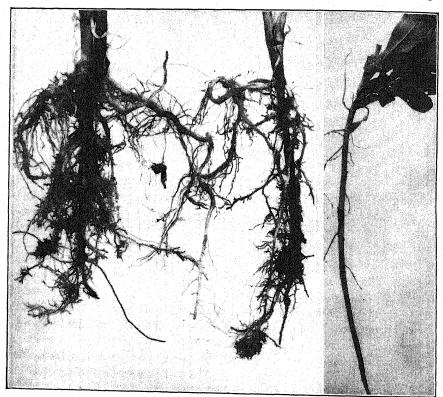


Fig. 1. At left, bearded root systems of two plants of corn, Zea mays L. (var. Hill's Yellow Dent) infected with numerous specimens of two different species of meadow nematodes (Pratylenchus sp.) and also a few specimens of the root-knot nematode (Heterodera marioni [Cornu] Goodey). Plants collected near Jacksonville, Texas, and submitted by P. A. Young. At right, a root of the goldenrod (Solidago leavenworthii T. & G.) with black lesions caused by a meadow nematode; specimen collected and submitted by H. G. Ukkelberg at Fort Myers, Fla.

by forming secondary roots and rootlets above the nematode lesions; a matted and bearded root system with only short feeder roots will result, i.e., a root system that exploits only the soil near the surface and close to the root crown, while deeper soil strata and more distant surface soils remain out of reach of the plant (Fig. 1). This condition shows on the above-ground parts of the affected plant in reduced growth, in wilting during the hot part of the day, in dying twigs or branches, in bronzing of leaves (evergreens) during

the winter, in winter kill, and in drought damage during the summer or what is sometimes called dieback or sunstroke. Various root crops are also liable to disfiguration by these meadow nematodes, particularly potatoes, peanuts (Fig. 2), and lily bulbs, in which instances the market value may be decreased or the product made directly unsalable.

The direct damage caused by these pests on roots is, however, only part of the story; all root-invading nematodes open the path for other, sometimes serious, secondary invaders which may result in necrosis of adjacent tissues and preclude recovery.

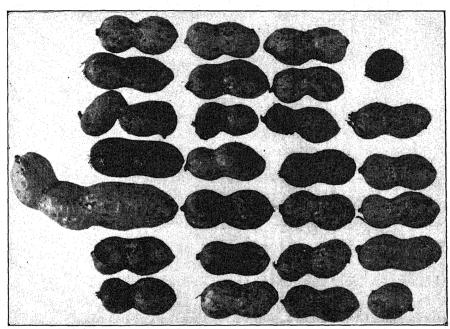


Fig. 2. Peanuts (Arachis hypogaea L.) disfigured by numerous black spots caused by a species of meadow nematode (Pratylenchus sp.); collected at the Substation at Holland, Va., of the Virginia Agricultural Experiment Station and submitted by D. J. Humphrey and L. J. Cushman.

The location and identification of pratylenchs in a root system or other plant organs necessitate a careful teasing apart of the tissues under a binocular microscope. High magnifications are necessary and identification of the species is difficult. Although the group as such is well characterized and appears to be a very natural one, its taxonomy has not yet been worked out. Nor is much known about the life cycle, host range, and geographical distribution of the various species of which at least five occur in the United States. There is need for more work on this group because of its economic significance.—G. STEINER.

A Bacterial Streak Disease of Phleum pratense L.1—A bacterial streak disease of timothy was first collected near Platteville, Wisconsin, in 1925.

¹ Journal Paper No. J-1283 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 450.

Later, in 1940, diseased timothy was collected in Mitchell County in the vicinity of Osage, Iowa, and near Kanawha, in Wright County, Iowa, along roadsides, in hay fields, and in pastures. In 1941 the disease was common in Story County around Ames, Iowa. It is difficult to estimate the loss to the timothy hay and forage crops caused by this disease because its prevalence fluctuates from year to year and is greatest during wet seasons.

The streaks of the disease varied from barely visible ones to those over two centimeters long on the leaf blades of the young shoots. A blotching of the whole leaf was common on the plants artificially inoculated in the greenhouse but was rarely observed in the field. When the necrosis of the leaf tissue became prevalent the plants were stunted. A striking symptom of the plants in the hay stage was the streaking on the blades and sheaths of infected flag leaves. Under severe attack the emerging heads were sealed in the spiral whorl by bacterial exudate or were malformed upon emergence

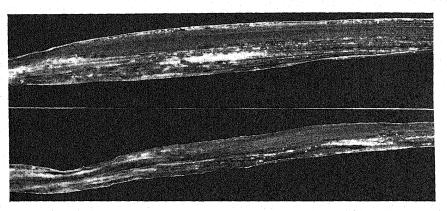


Fig. 1. Water-soaked, translucent streaks on timothy leaves artificially inoculated with the timothy strain of Xanthomonas translucens.

from the boot. During warm humid days, yellowish droplets of bacterial exudate formed on the surface of the lesions even as late as October and November. Upon drying, these droplets formed hard, resinous granules. In the later stages of the disease the affected tissues became dry and brown, but even these older brown lesions were translucent by transmitted light. The symptoms of this disease were very similar to those caused by Xanthomonas translucens on barley, brome grass, rye, and wheat.

A yellow bacterium was isolated from the translucent, water-soaked streaks and from the dry, brown lesions. The bacterium was a Gram-negative, monotrichous rod averaging 0.5 to 0.8 μ by 1.5 to 2.0 μ , depending upon the age of the culture studied. In culture the organism was a yellow waxy color. Colonies on nutrient agar were raised, with a smooth glistening surface. After positive proof of pathogenicity, the comparative cultural characteristics of three isolates of the causal agent were studied. All grew slowly in beef-peptone agar, while on potato-dextrose agar growth was copious and a watery, creamy yellow with whitish margins. In nutrient

broth the organism formed a coarse pellicle. The organism liquified gelatin, but did not reduce nitrates, produced hydrogen sulphide, produced ammonia, reduced the indicator and produced an alkaline reaction in litmus milk, did not produce acetyl-methyl-carbinol, was methyl-red-negative, did not utilize citrate, showed diastase activity, utilized only dextrose of the seven monosaccharides tested in inorganic basal medium and utilized the disaccharides maltose and sucrose. Culturally and biochemically the organism was like *Xanthomonas translucens* although there was some variation between isolates of the timothy strain and other strains of *X. translucens* in utilization of carbohydrates.

After identifying the bacterium, repeated cross inoculations were made to determine whether or not the bacterium was a new variety of Xanthomonas translucens. The organism caused typical symptoms on timothy (Fig. 1) by both the spray and hypodermic methods of inoculation, but it did not infect barley, brome grass, oats, rye, or wheat. As a result of these trials the bacterium causing a bacterial streak disease on timothy is designated a variety of Xanthomonas translucens as follows: Xanthomonas translucens var. phleipratensis var. nov. Isolated from Phleum pratense. Produces water-soaked lesions at 25° to 30° C. on shoots of Phleum pratense following wound inoculation.—J. R. Wallin and C. S. Reddy, Iowa Agricultural Experiment Station, Ames, Iowa.

Notes on Physiologic Specialization in Puccinia graminis tritici Erikss. and Henn. in China. In 1932, Tu² reported the occurrence of 6 physiologic races of Puccinia graminis tritici in China. As far as the writer is aware, it is the only published record regarding physiologic specialization of wheat stem rust in this country.

During 1942–1944, the writer made 175 collections of *Puccinia graminis tritici* from 12 provinces in China. Inoculations on the 12 standard wheat varieties,³ seed of which was obtained through the courtesy of Dr. E. C. Stakman of the U. S. Department of Agriculture and the University of Minnesota, U.S.A., demonstrated 2 new physiologic races and 12 already known races, *viz.*, Nos. 10, 11, 15, 34, 39, 40, 95, 107, 115, 122, 143, and 189.⁴ Races 15, 107, and 122 are by far the most common: they have been identified 35, 44, and 24 times, respectively, out of 175 collections. Race 122 is the most widely distributed, occurring in 9 of 12 provinces.

The two new races are tentatively numbered C₁ and C₂. The reactions

The writer is indebted to Dr. E. C. Stakman for reading the manuscript.

² Tu, Chih. Physiologic forms of *Puccinia graminis tritici* in Kwangtung, Southern China. Phytopath. 24: 423-424. 1934.

³ Stakman, E. C., and M. N. Levine. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Stat. Tech. Bull. 8. 1922. (Mimeographed keys and tables for identifying races are available.)

⁴ Garcia-Rada, G., J. Vallega, W. Q. Loegering, and E. C. Stakman. virulent race of wheat stem rust No. 189. Phytopath. 32: 720-726. 1942.

¹ Phytopathology extends the courtesy of its journal pages to scientists in other countries who are persevering in research under difficult wartime conditions and are temporarily deprived of the opportunity for membership in the American Phytopathological Society.

of the standard wheat varieties to these races were tested 5 times at temperatures ranging from 22° to 28° C., and the results obtained were consistent (Table 1).

TABLE 1.—The infection types produced on 12 standard differential wheat varieties by races C_1 and C_3 of Puccinia graminis tritici

			Inf	ection	ı typ	es on	whea	at ho	stsa			
Acc. Place of No. collection	p	Ma	Krd	Ko	Arn	Mnd	Spm	Kub	Ac	Enk	Ver	Kpl
C ₁ Kaiyüan, Yunnan Kunming, Yunnan	1++	2++	4++	0;	4++	4++	4++	4++	4++	3+	1++	3+c
C ₂ Hwayang, Szechuan Chengtu, Szechuan	4+	3±°	4+	0;	4++	4+	4+	4++	4+	4-	3	1+

^a The wheat hosts are those used by Stakman and Levine to determine physiologic races of wheat stem rust.

Race C₁ was first isolated from common wheat in Kaiyüan and then again from Khapli emmer in the nursery. It can be differentiated from known races by the infection types it produces on Kota and on Vernal. It is similar to races 41 and 42 of Stakman and Levine except on Kanred, on which this new race produces a 4++ infection type instead of the 0 infection type. Race C₁ also differs from races 72 and 99 by its effects on Little Club and Arnautka. Race C₂ is similar to race 122 except that the former produces a type 3 infection and the latter a type 1 infection on Vernal.—Sin-Yün Yin, Division of Plant Pathology, Institute of Agricultural Research, National Tsing Hua University, Kunming, Yunnan, China.

A Graft-Transmissible Mosaic Disease of Grapevine.—A mosaic type of disease reported to be killing grapevines (Vitis vinifera) in a vineyard in Napa Valley, California, was brought to the writer's attention in the summer of 1943. The disease involved a somewhat circular area of about 60 vines near the center of a block of the variety Palamino grafted on St. George root stock.

The leaves of diseased vines from emergence to leaf fall had varying degrees and patterns of chlorosis that consisted essentially of yellow, cream, and light green areas. The cream chlorosis occurred as a narrow band along the smaller veins (Fig. 1, A), as irregular blotches along the large veins (Fig. 1, B and D), or as a stippling or spattering over the leaf surface (Fig. 1, E). Some leaves were entirely yellow with only traces of green along the large veins. In other leaves (Fig. 1, F) the light green chlorosis appeared to have originated in the veins and spread, as if leaking from the veins, into the surrounding tissue. Many leaves had two or more of the types of mottling with one type usually predominating. An occasional leaf appeared to be covered with a mixture of types and had some veinlet clearing as well (Fig. 1, C). The cream and yellow mottled areas were usually

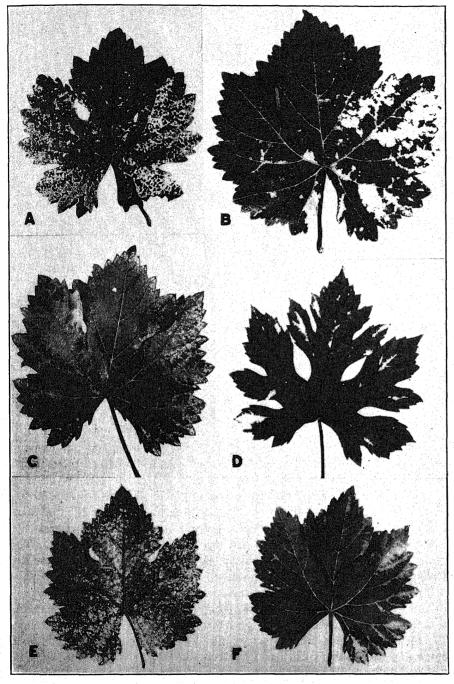


Fig. 1. Leaves of Vitis vinifera with different patterns of mottling associated with a graft-transmissible mosaic. A. Leaf with cream-colored bands along the small veins and also some necrosis of the basal lobes. B. Leaf with irregular creamy blotches, mostly along the veins. C. Leaf with a combination of cream, yellow, and light green mottling and also some veinlet clearing. D. Leaf from a seedling vine inoculated by grafting a bud from a diseased vine into the stem. The leaf has cream-yellow patches along the large veins. E. Leaf in which the yellow color appears to have been stippled over the surface. F. Leaf with light green areas along the veins.

faint and indistinct in the young leaves, but as the leaves matured the areas became prominent, with definite margins contrasting with the gradiant the leaf. During the summer and fall the mottled areas in the leaves usually faded to light cream or nearly white, and parts of the leaves dried.

In September, 1943, twenty-five cuttings were taken from diseased vines in the Napa Valley vineyard and rooted in sterile soil. Sixteen of the cuttings grew and all of these had mottled leaves. At the same time, buds were also taken from diseased vines and grafted, one each, into 5 healthy seedling vines growing in greenhouse pots. By January, 1944, the stock wood of 3 budded vines had developed mottled leaves typical of those of the diseased vines from which the buds were originally taken. Again, in 1944, buds from diseased vines were grafted into 10 additional healthy seedlings and 15 healthy rooted cuttings of the variety Palamino. During the fourth and fifth months of incubation, the stock growth in 7 of the 10 seedling vines and 12 of the 15 Palamino rootings had developed mosaic leaves. Check plants of seedling vines and Palamino rootings handled in the same manner remained healthy. These experiments show that this mosaic disease of grapevines is graft-transmissible.

During July, 1944, the carborundum method described by Rawlins and Tompkins, was used to inoculate 25 separate seedling vines with juice extracted from chlorotic leaves. After 8 months of incubation, none of the juice-inoculated plants had developed leaf-mottling symptoms.

This graft-transmissible mosaic disease of grapevines found in California resembles very closely the juice-transmissible mosaic of Vitis vinifera in central Europe described by Strănák et al.2—Wm. B. Hewitt, Division of Plant Pathology, University of California, Davis, California.

A More Virulent Black Pit Organism on Citrus.—In some lemon and orange groves of California large spots (1.5 inch diameter) were found on lemon and Valencia orange fruits (Fig. 1, C) either solitary or associated with the typical black pit caused by Phytomonas syringae (Bacterium citriputeale, Bact. citrarefaciens). These spots, observed for the first time in 1945, averaged much larger than the usual black pit of lemon and suggested either a greatly increased virulence or a different organism. tissues were depressed, brown, and had a sharply defined darker margin. Some of these spots were evidently caused by the coalescence of the smaller black pit lesions, while in other spots no coalescence could be observed.

Cultures made from the typical black pit lesions and from the margins of the larger spots gave bacterial growth typical of the black pit organism. On inoculation, these cultures caused the development of spots reaching a maximum of 1.5 inches (35 mm.) in diameter (Fig. 1, B) rather than the usual small black pits (Fig. 1, A). The maturity of the fruit and the viru-

¹ Rawlins, T. E., and C. M. Tompkins. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. 26: 578-587. 1936.

² Stranak, F., Ctibor Blattný, and A. Klečka. Mosaika révy vinné. Ochrana Rostlin. 11: 89-98. 1931.

lence of the organisms are believed to determine the size of spots. Since spots of this size had not previously been found in nature, the recent isolates appear especially virulent. Smith¹ in his description of the black pit of

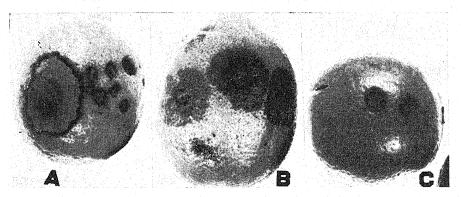


Fig. 1. Black pit on lemon: A, Natural infection showing the large spot with the darker margin and small typical black pits; B, Artificial inoculation showing the large spots before the tissue has become depressed; C, Natural infection on Valencia orange.

lemons gave the size of the lesions as 5-20 mm., with larger lesions rarely occurring.—Clayton O. Smith and L. J. Klotz, University of California Citrus Experiment Station, Riverside, California.

1 Smith, Clayton O. Black pit of lemons. Phytopath. 3: 277-281. 1913.

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L. M. Massey

R. S. Kirby, Chairman Botany Department State College, Pennsylvania

THE RED-SPOT DISEASE OF BROAD BEANS (VICIA FABA L.) CAUSED BY BOTRYTIS FABAE SARDIÑA IN CHINA¹

T. F. YU

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INTRODUCTION

A leaf-spot disease of broad bean (Vicia faba L.) was noticed early in 1925 by Dr. R. H. Porter, who was, then, the plant pathologist of the University of Nanking, China. At his suggestion, investigations on the nature and cause of the disease and possible remedial measures were begun in the spring of 1927. Two years after this work was started, Sardiña (20) published on a leaf-spot disease of broad bean in Spain and attributed it to a new species, Botrytis fabae. The disease found in China is identical with that reported by Sardiña in Spain.

The work described in this paper was done from 1927 to 1929 and again from 1934 to 1937 in the Plant Pathology Laboratory, the University of Nanking, Nanking, China. The war stopped the work in November, 1937, and no opportunity of continuing the investigation or of publishing the results has occurred until now.

The name red spot was adopted in order to distinguish this disease from the chocolate spot of the same host which, according to Wilson (24) in England, is caused by *Botrytis cinerea* Pers.

HISTORY OF THE DISEASE

The red-spot disease has undoubtedly been present in many of the bean-growing regions for a long time. Early records of the chocolate-spot disease of broad bean attributed that disease to *Bacillus lathyri* Ma. and Taub. (14, 15, 17, 18, 19), but it has lately been demonstrated that *Botrytis cinerea* Pers. (25) and also *B. fabae* Sardiña (5, 8, 11) may cause chocolate spot.

The first authentic report of the red-spot disease was that by Sardiña (20) in 1929, in which the causal fungus was described as a new species of Botrytis (Botrytis fabae Sardiña). His second report appeared in 1931 (21) and two diseases of broad bean were described, one of which was at tributed to B. fabae Sardiña, the other to a form of B. cinerea Pers. Ir these two papers the symptoms and etiology of the red-spot disease and the physiology and cultural characters of the pathogen were fully described

In 1933, Ikata (8), probably without the knowledge of Sardiña's work reported from Japan the existence of a red-spot disease of broad bean caused by a new species of *Botrytis* which has also been named *B. fabae* n. sp. He described, in detail, the morphological and cultural characters of the pathogen as well as the control measures with fungicides.

¹ Paper No. 66, Plant Pathology Laboratory, the University of Nanking, Chengtu, China.

Other reports pertaining to *Botrytis* disease of beans were later given by Nattrass (11, 12, 13), Montemartini (10), Berger (3), El-Helaly (5), and Chorin (4).

DISTRIBUTION AND ECONOMIC IMPORTANCE

Red-spot disease of broad bean caused by *Botrytis fabae* Sardiña is widely distributed in the bean-growing regions throughout the world. It has been reported from Spain (20), Japan (8), England (24), Cyprus (11), French Morocco (3), Italy (10), Palestine (4), and Burma (17).

In China, it was first recorded by Porter in 1925 (see Annual report for the year 1924–25, College of Agriculture and Forestry, The University of Nanking, Nanking, China, 1926). He gave a brief report of its occurrence in Southeastern China and referred to it as "red spot" of broad bean. Surveys made by the writer from 1934 to 1937 inclusive revealed the wide distribution of the disease, especially along the Yangtze Valley and near the sea coast where it was observed in almost every field. In the inland regions, however, it was much less prevalent, while in the southwestern corner it was rare. Diseased specimens were also received from as far north as Hopeh province. In general, the disease seems to be co-existent with the crop throughout the country.

No exact figures are available regarding the amount of damage from redspot disease of beans. The severity of the disease is correlated with weather conditions. In dry regions such as Yunnan of southwestern China, where the average monthly precipitation during March and April seldom exceeds 45 mm., the disease appears only as a few small spots on the leaf blades throughout the season. Consequently, it is of no economic importance. On the other hand, in regions where the atmospheric humidity is high, heavy losses to the crop result. The most severe outbreaks were observed along the river and near the sea coast, and there the disease is not only very prevalent but also destructive. Severe defoliation of bean plants and blight in the stems were seen in some of the worst cases. No experimental work had been done in determining the cause of stem blight, but Botrytis cinerea Pers. and Bacillus fabae were known to be capable of inducing blight in stems (26). The bacterial pathogen, a wound parasite, is very active in emphasizing blight and rot in stems under favorable conditions.

SYMPTOMS OF THE DISEASE

Red-spot disease of broad bean is most common on the leaves during the spring. The first signs of the disease are minute red spots on the leaf blade. As they enlarge, the center becomes depressed and the color changes to a chocolate or iron red with deep-colored margin. The spots are round, oval, or sometimes oblong and are generally numerous on a single leaf. Most of the spots are less than 1 mm. in diameter and usually remain small throughout the season. Their size probably is determined by the number per unit area. Spots as large as 4 or 5 mm. are also present. Isolations made from the large zonate spot yielded a typical culture of *Botrytis fabae*

Sardiña, and upon reinoculation with its spores on broad bean the characteristic small red spots were produced almost exclusively. Two or more spots may coalesce to form larger irregular lesions. The tip and margin of the leaf blade become black and papery and die. The dead tissues are easily cracked or torn, while the remainder of the leaf, although it bears numerous spots, may be a normal green for a time. Under moist conditions, the dead areas are soon covered with a light gray coating of conidiophores and With continued moist weather, there is still further development of the disease. The entire leaf blade may be involved and may collapse, although it remains attached. Excess moisture in the field results in the It is also not uncommon to find the plants defoliated, esperot of the leaf. cially in a thickly sown field. Infection occurs mostly on the upper leaf Spots on the lower leaf surface are few and large and have an indistinct margin. Long red stripes are also seen on the lower leaf surface. Microscopic examination of the affected leaf tissues shows that the mycelium of the fungus is intercellular.

Lesions on petiole and stem are oblong, elliptical, and deeply sunken at the center with a deep red margin. Their size varies considerably depending on the size of the affected plant part. On large stems, lesions may be 1 cm. long.

On pods, the fungus occasionally produces very tiny red spots which remain small throughout the season. No apparent damage from the fungus has ever been observed on the pods.

ISOLATION OF THE FUNGUS

The fungus is very easy to isolate. When any surface-disinfected portion of the diseased plant bearing the spots is placed upon a poured plate of potato or corn meal agar at ordinary room temperature, the fungus will develop rapidly. At first the colony consists of dirty white mycelial growth only, but after a few days spherical white bodies, which soon turn shiny black, appear at the center of the colony, and finally a large number of them form over its surface.

THE CAUSAL FUNGUS

Red-spot disease of broad bean in China is caused by *Botrytis fabae* Sardiña.

The hyphae are septate, branched, and usually coarse, both in lesions and in cultures. Dark-colored appressoria are commonly formed in culture by the branched hyphae which come in contact with glass containers.

Sclerotia are commonly found in cultures but so far have not been observed upon the host in nature. Newly formed sclerotia in cultures appear as white masses of mycelium. As development continues, the surface becomes waxy and light olivaceous green, then gradually darkens, until a hard black mass results. Mature sclerotia are round, oblong, or irregular. When disinfected diseased leaves are incubated in moist Petri dishes, round or very slender sclerotia are formed in great abundance along the leaf vein.

On ordinary culture media at room temperature, sclerotia are $0.5-3\times0.6-3.5$ mm. with an average of 1×1.5 mm. The size of sclerotia is greatly influenced by temperature and by composition of the substratum. Sclerotia may be in rings or scattered on the surface of agar in dishes. It is not uncommon to find them aggregated into crusty masses. They germinate either by sending out mycelium or by producing conidiophores and conidia upon their surfaces. Direct germination of sclerotia, however, has been less common throughout the present investigation.

Conidia may be produced directly from fine mycelium or from sclerotia. They are septate, twisted, and from 8 to 20 μ in diameter and 600 to 1500 μ in length. The growing tip of the conidiophore sends out several side branches from the main stem and these, in turn, branch. The ends of these branches swell and send out the sterigmata on which lateral conidia are produced. Conidia are produced profusely in clusters that are either terminal or intercalary.

Conidia are subglobose to oval, hyaline, and 12.2–22.8 \times 10.5–15.8 μ with an average of 16.7 \times 13.7 μ . Under favorable conditions they germinate in a few hours by sending out one or two germ tubes from each end.

Microconidia are abundant on culture media, especially media poor in carbohydrate. They are hyaline, globose, or oval and 2.9–4 μ in diameter with an average of 3.6 μ . No germination of microconidia has ever been observed. Considerable variation is noticeable in the type of conidiophore. The most frequent form is a cluster of conidiophores arising directly on a cell of the sterile hyphae. In another type a special branch arises from the sterile hyphae. In still another form a long slender branch bears upon its tip either a single conidiophore or a group of conidiophores. Microconidia originate from at least three sources, mycelium, germinating conidia, and germinating sclerotia. It is interesting to note that sclerotia, when placed on an agar surface after a certain period of desiccation, will germinate by producing an extremely slow growing mycelium. Small white and green mycelium-like tufts are scattered over the agar surface. These are the clusters of microconidia.

PHYSIOLOGY OF THE CAUSAL FUNGUS

Cultural Studies

In studying the cultural characters of *Botrytis fabae*, both natural and artificial culture media were used. No attempt is made to give detailed descriptions of the growth characters of the fungus on each kind of medium. In general, it thrives well on all the media commonly used in the laboratory. It grows rapidly and forms a fluffy mycelium which later becomes appressed to the surface of the agar and has a glistening appearance, and sclerotia then begin to form. Aerial mycelium is scant and is usually at the top of an agar slant. Sclerotia and microconidia are usually present. Conidia, although formed abundantly in nature, are produced only occasionally in artificial media in the laboratory. It has been pointed out repeatedly by

the previous investigators that the production of conidia is influenced by the amount of moisture in the air (7, 9, 25). Attempts were made to induce production of conidia, but the partial drying of plate cultures described by Hopkins (7) produced only inconsistent results. Undoubtedly the production of conidia is largely a matter of moisture relations, yet the possible influence of other unknown factors must also be considered.

Temperature in Relation to Growth of Mycelium

The relation of temperature to the rate of mycelial growth on plates of potato-dextrose agar was studied. Separate dishes were inoculated with the fungus and placed in constant temperature chambers, adjusted to temperatures between 5° and 36° C. Two experiments were made. In the first experiment, abundant mycelial growth occurred between 20° and 27° C. within four days. In the second experiment, cultures incubated at 24° to 26° C. grew better than those at 15°–17° C. or at 30° C. in the same length of time. The optimum temperature for mycelial growth of the fungus lies somewhere between 24° and 26° C. (Table 1). Cultures incubated at 5° to 7°

TABLE 1.—The relation of temperature to growth of mycelium of Botrytis fabae $Sardi\~na$

Experime	nt 1 (4 d	lays)	Experiment 2 (4 days)				
Temperature, in degrees C.		Diameter of the colonya	Temperature, in degrees C.	Diameter of the colonya			
		cm.		cm.			
19-19.6		3.8	15-17	2.3			
20-20.4		5.3	24-26	5.6			
21-22		6.7	30-34	2.1			
25-25.3		6.9					
27-28		6.1					

a Average of ten plate cultures.

C. produced slight growth by the eighth day, and on the 17th day the colonies had attained diameters from 0.5 to 2 cm. and sclerotia were appearing on the surface. At 36° C., very little growth was seen for a long time. The minimum temperature for mycelial growth, therefore, probably is about 6° C. and the maximum about 36° C.

Relation of pH to Growth of Mycelium

The effect of pH of culture medium on the rate of mycelial growth was determined from the dry weight of the mycelia produced in the standard nutrient solution incubated at 24° C. for seven days. Determination of the pH was by both the colorimetric and electrometric methods. The average dry weight of the mycelium, including few small sclerotia, of ten flasks for pH 3.2, 4.4, 5.2, 6.2, 8.1, and 8.5 were respectively 0.00, 0.25, 0.26, 0.14, 0.13, 0.12, and 0.00 mgm. The optimum pH for mycelial growth lies between 4.4 and 5.2. El-Helaly (5) found that the fungus thrives best at pH 4.5,

while Sardiña (21) found the optima for mycelial growth of two strains of the fungus at pH 5.3 and at pH 7.3.

Germination of Conidia

Germination of conidia of *Botrytis fabae* was tested at various temperatures. A spore suspension was made in tap water and drops were transferred to clean glass slides placed in moist Petri dishes in the incubators. In the first test the conidia germinated best between 20° and 25° C. within 24 hours (Table 2). If the duration of the test was prolonged, the conidia germinated over a wide range of temperatures. In a second test, attempts

TABLE 2.—Relation of temperature to germination of conidia of Botrytis fabae Sardiña

Experiment	1 (24 hours)	Experiment 2 (48 hours)						
Temperature, in degrees C.	Percentage of germinating conidia	Temperature, in degrees C.	Percentage of germinating conidia					
 10-11	0.0	15–17	76.8					
14-15	11.1	17-19	85.4					
20-21	68.9	19-21	93.0					
24-25	58.9	22-23	74.1					
29-30	23.6							

were made to determine more exactly where the optimum temperature lies. It is between 19° and 21° C. This optimum is close to that found by Ikata (8) who reported it at about 20° C. The maximum and minimum temperatures for conidium germination were 34° and 5° C., respectively.

HOST RANGE OF THE FUNGUS

In inoculation tests, Sardiña (21) found that Botrytis fabae is unable to infect plants outside the Leguminoseae. Among the leguminous plants, he obtained infection on French bean. Ikata (8) inoculated Vicia faba, Pisum sativum, Phaseolus vulgaris, Astragalus sinensis, Medicago sativa, and a second species of Vicia with a suspension of conidia of the fungus and found none of these plants, except its own host (V. faba), was infected. Schnellhardt and Heald (22), in connection with their studies on the decays of apple, made artificial inoculation on the fruit with various species of Botrytis including B. fabae Sardiña. This fungus was only weakly parasitic to apples. Nattrass (12) observed a chocolate-spot disease of Vicia sativa in nature. The sporulation of its causal fungus closely resembled that of B. fabae Sardiña and when broad beans were artificially inoculated with a suspension of conidia, the characteristic chocolate spots were produced.

In the course of the present investigation, inoculation experiments were repeatedly made for determining the host range of the fungus. The results obtained indicate that *Botrytis fabae* will not attack non-leguminous plants such as potato, tomato, pepper, eggplant, lettuce, carrot, celery, radish, and cabbage. Inoculations with suspensions of conidia on the following

leguminous plants also gave negative results: Arachis hypogaea L., Cercis chinensis Bunge, Dolichos lablab L., Glycine max Merr., Indigofera spp., Lespedeza bicolor Turcz., L. striata Hook. & Arn., Lupinus albus L., Medicago sativa L., Melilotus alba Desr., M. officinalis (L.) Lam., Phaseolus angularis Wight, P. aureus Roxb., P. mungo L., P. vulgaris L., Robinia pseudoacacia L., Sophora japonica L., Trifolium incarnatum L., T. pratense L., T. repens L., Vicia tetrasperma (L.) Moench., V. villosa Roth., Vigna sinensis Savi., Wisteria sinensis Sweet. The leguminous plants that were infected were Vicia faba L., Pisum sativum L., P. sativum var. arvense Poir., and Vicia sativa L. The fungus invariably produced small red spots on Vicia sativa L. when it was artificially inoculated. In the case of Pisum sativum L. and P. sativum var. arvense Poir., the results obtained were variable. In a few tests, the fungus failed to infect the plants.

OVERWINTERING EXPERIMENTS

Experiments were undertaken to determine by what means the fungus lives through the winter. Sclerotia produced on sterile soybean stems in flasks were placed in a wire basket and left in a bean field where they were exposed to prevailing weather at Nanking from November to March in the winters of 1928, 1934, and 1935. Sclerotia were viable at the end of the winter in all three years.

In another experiment, sclerotia taken from potato-dextrose-agar plates were either left on the soil surface in the field or buried about two cm. below the soil level in pots containing fine clay soil. The experiment was started Nov. 1, 1934. Forty days later, abundant sporulation was seen on the surface of sclerotia which had been left on the soil surface. None of the buried sclerotia had germinated at the end of 68 days. They were then turned and brought to the soil surface, and there was abundant sporulation about a month later. These experiments indicated conclusively that sclerotia may carry the disease through the winter.

In order to find out whether or not conidia might live over the winter, experiments of the viability of conidia under desiccation were made. Drops of spore suspension made of fresh spores were placed on clean glass slides in a dry container hanging on a tree in the field and left under drying conditions. Germination tests were made at regular intervals until no germination was observed. About 80 per cent of the spores germinated at the end of 70 days. After this, the percentage of spore germination dropped sharply to about twenty. At the end of 140 days, few spores still retained their viability. Since the broad bean crop is harvested in the middle of June and the disease has never been observed in November under natural conditions, it is improbable that conidia produced on the affected plants of the first year cause infection in the spring of the next year.

LIFE HISTORY STUDIES

It has been stated above that sclerotia have never been observed on the diseased plants under natural conditions. However, when affected stems

were cut and piled in a low moist shady place in a field, sclerotia were occasionally found either on the surface or underneath the epidermis of the stems during July or August. In this connection, it is interesting to mention that in certain inoculation experiments, sclerotia had been found underneath the epidermis of the inoculated plants that were kept under moist conditions for a considerable time. The writer is inclined to believe that the production of sclerotia under natural conditions, if it occurs, is determined by proper moisture and temperature conditions as well as by the stage of maturity of the host plants.

In the overwintering experiments, the fungus survived the dormant period as sclerotia. Sclerotia germinate in the spring and produce abundant conidia which bring about primary infection. This primary infection is tremendous because sclerotia may produce several crops of spores in a short time. In one laboratory experiment, sclerotia with conidia taken in from the fields on March 22 were washed thoroughly with tap water and incubated in moist dishes. Conidia were again formed in less than 24 hours. The sclerotia were washed again, dried, and again washed. The procedure has been repeated as many as six times in 24 days and conidia were still produced by the sclerotia.

Under favorable conditions, conidia produced in great abundance on the first-infected leaves furnish abundant inoculum for the second infection, which occurs commonly in May. A great number of spores were caught on dishes containing agar or on slides coated with vaselin when these devices were exposed in a bean field. The prevailing high atmospheric humidity is extremely favorable for production of conidia and for infection by the fungus.

INFLUENCE OF CLIMATIC CONDITIONS

Red-spot disease of broad bean has been found in all the bean-growing districts along the Yangtze river and near the sea coast of China, but it is rare in many other regions of the country. Diseases caused by *Botrytis* are generally prevalent and severe under high moisture (1, 2, 6, 16, 23, 25). In case of the present disease, Sardiña (21) found that a relative humidity of at least 85 per cent is necessary to induce infection on beans. Experiments by the writer also indicated that a relative humidity of 90 per cent or above is essential for infection as well as for production of conidia on affected plant parts.

The influence of moisture on this disease may be better understood by comparing meteorologic data, for the two months prior to the harvest of the crop in a dry and a wet region. They may be represented by Kunming in southwest China and Nanking along the river. In Kunming, the disease appears rarely, whereas in Nanking it occurs in great abundance. The average monthly precipitation in Kunming for March and April in the five years from 1928 to 1933 were respectively 38 and 47 mm. Relative humidities for the same months were respectively 60.5 and 61.8 per cent, amounts

far below the minimum as shown both by Sardiña (21) and the writer. In Nanking, the average monthly precipitation for April, May, and June in 1933 was respectively, 114.1, 116.4, and 135 mm., and the relative humidity for the same months was respectively 83.2, 85.2, and 93.1 per cent. difference in moisture conditions apparently explains the incidence of the disease in these two regions.

SUMMARY

A leaf-spot disease of broad bean in China, here referred to as red-spot disease, is found to be identical with that described by Sardiña in Spain and is caused by Botrytis fabae Sardiña. The disease is widely distributed in China, especially in the bean-growing regions along the Yangtze river and near the sea coast. Under suitable environmental conditions, affected leaves may collapse or plants may be so defoliated that pods do not form.

The symptoms of the disease and the morphology and physiology of the causal fungus have been described.

The fungus appears to be limited to a few species of the Leguminoseae. Of a large number of leguminous plants exposed to artificial inoculation, only Vicia sativa L., Pisum sativum L., and P. sativum var. arvense Poir., in addition to Vicia faba L., were slightly infected. The fungus so far has been unable to attack plants outside the Leguminoseae.

Overwintering experiments indicate that the fungus may overwinter by means of sclerotia, although they have never been observed on diseased plants in nature. The disease is probably not carried through the winter by conidia produced on the affected plants of the previous season.

The prevalence of red-spot disease in the various bean-growing regions is correlated with rainfall and atmospheric humidity. The scarcity of the disease in the southwest is correlated with the dry climate whereas its common occurrence along the river or near the sea coast is correlated with high atmospheric moisture during the two months prior to the harvest of the beans.

No attempts have been made to control the disease.

University of Nanking,

CHENGTU, CHINA.

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DISTRIBUTION OF RACES OF TILLETIA CARIES AND TILLETIA FOETIDA AND THEIR RELATIVE VIRULENCE ON CERTAIN VARIETIES AND SELECTIONS OF WHEAT¹

H. A. RODENHISER AND C. S. HOLTON²

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INTRODUCTION

Experiments were started in 1932 to obtain information on the number, distribution, and prevalence of physiologic races of Tilletia caries (D. C.) Tul. (T. tritici (Bjerk.) Wint.) and T. foetida (Wallr.) Liro (T. levis Kuehn) in the different wheat-growing regions of the United States. In previous papers (4, 7) 14 races of T. caries and 10 of T. foetida were described on the basis of the reaction of certain differential varieties of winter and spring wheats. These data, together with information on the prevalence and distribution of the races, are basic to a well-rounded program for the development of bunt-resistant varieties. Data are presented here (1) on the identification of 7 previously undescribed races, (2) on the distribution of species and races in the principal wheat-growing areas of the United States, and to a limited extent in Mexico, and (3) on the relative virulence of races on certain winter and spring wheat varieties and hybrids that may be of interest to wheat breeders in connection with the development of bunt-resistant varieties.

NEW PHYSIOLOGIC RACES

The methods used in the identification of bunt races have already been described (4, 7). The reactions of differential wheat varieties to all known races, including 2 new races of Tilletia caries (T-15 and T-16) and 5 of T. foetida (L-11 to L-15, inclusive) are in table 1. The original source of each of the 7 new races and the distinguishing pathogenic characters of each are shown in table 2. Among the races previously described, there are several that differ from each other in relative degrees of virulence on the same differential hosts. This is likewise true of 6 of the new races here described and their occurrence probably does not change greatly the factors to be considered in breeding for bunt resistance. However, the new races T-15 and T-16 complicate the breeding program somewhat since one may no longer expect to obtain varieties from combinations of Hohenheimer, Hussar. and Oro that are resistant to all known races. It should be possible, however, to obtain the desired resistance by bringing in the so-called Ridit factors which control resistance to both of these races.

² Senior Pathologist and Pathologist, respectively, Division of Cereal Crops and Diseases.

¹ Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Agricultural Experiment Stations of Washington and Idaho.

TABLE 1.—Reactions of differential wheat varieties to physiologic races of Tilletia caries and T. foetida

Species and race	Hybrid 128	Ridit Oro	Hohenheimer	Hussar	Albit	Martin	White Odessa	Ulka	Marquis	Canus
T. caries T-1 T-2 T-3 T-4 T-5 T-6 T-7 T-8 T-9 T-10 T-11 T-12 T-13 T-14 T-15 T-16		R R R R R R R R R R R R R R R R R R R	RERERERESESERSS	R R R R R R R R R R R R R R R R R R R	RRRIISSSRRRSSSR	RRRSSSSSRRRIRRSR	RRRSSSSSRRRSSSSR	авававававнанава	IRSSSSSSIISRSSI	RRSRSRISRRSRIRSI
T. foetida L-1 L-2 L-3 L-4 L-5 L-6 L-7 L-8 L-9 L-10 L-11 L-12 L-13 L-14 L-15		R R R R R R R R R R R R R R R R R R R	R R R R R R R R R R R R R R R R R R R	R R R R R I S R I I R R R R R R S R S R	RRRSSSSRSRRSRSS	R R R S S S S R R R R R R R I I I	R R R R S S S S R S R R R R R S S S	aaaaaaaaaaaaaa	ISSISSISHIIISSS	RRSRSSSSSIRRRI

^a R=Resistant (0-10 per cent infection); I=Intermediate (11-40 per cent infection); S=Susceptible (41-100 per cent infection).

Races L-13, L-14, and L-15 were identified from the collections made in Mexico (Table 2). L-13 is the only race of *Tilletia foetida* that is pathogenic on the variety Hohenheimer. In this respect it is similar to T-10, but the two differ in pathogenicity on Ulka and Marquis. Races L-14 and L-15 have the same host range as L-4 and L-7, respectively. The distinction between L-14 and L-4 is based on the degree of virulence on Martin and Marquis, while L-15 and L-7 differ in degree of virulence on Marquis and Canus.

DISTRIBUTION OF SPECIES AND RACES

Species

Surveys to determine the distribution of *Tilletia caries* and *T. foetida* have been made by several investigators (2, 6, 13). It is evident from their

1945]

reports that *T. caries* occurs over a considerable area of the United States but its prevalence is most marked in certain regions. It is common in the durum wheats grown in the Upper Mississippi Valley and greatly increases in relative prevalence toward the intermountain area of the Northwest where it has been the predominating species. Elsewhere in the United States *T. foetida* has always predominated and in recent years it has increased in relative prevalence in the Northwest. In fact, according to Kienholtz and Heald (6), only *T. caries* was known to occur in the State of Washington prior to

TABLE 2.—Original source and differential characteristics of seven new races of Tilletia caries and T. foetida

Species and race	Collec- tion No.	Source and differential characteristics
T. caries		
T-15	571	From Oro wheat in 1938 near Cottonwood, Idaho, collected by W. M. Bever, who made preliminary tests on differential hosts and reselected it from Hohenheimer at Moscow, Idaho. It is similar to T-12 on several varieties but differs from this race by the susceptibility of Hussar, Martin, Marquis, and Canus.
T-16	346	From a field of Leap wheat in 1938 near Charlestown, W. Va. It is characterized by the susceptibility of Oro and Hohenheimer and the intermediate reaction of Marquis and Canus. It is the only race that is pathogenic on both Oro and Hohenheimer.
T. foetida		
L-11	264	From a field of mixed wheat in 1935 near Sutter, California. It is similar to race L-1 and differentiated from it only by the reaction of Canus which is resistant to L-1 and intermediate to L-11
L-12	278	From a field of Poole Wheat in 1936 near Staunton, Va. It is characterized by the susceptibility of Albit and the resistance of all of the other winter wheat differential varieties.
L-13	308	From a field of mixed wheat in 1936 near Mexico City, Mexico It is characterized by the susceptibility of Hohenheimer. This is the only known race of the species to which this variety is susceptible.
L-14	307	From a field of mixed wheat in 1936, 30 miles northwest of Mexico City, Mexico. It is similar to race L-4 but differentiated from it by the intermediate reaction of Martin and the susceptible reaction of Marquis.
L-15	311	From a field of mixed wheat in 1936 near San Juaquin, Mexico This race is similar to L-7 but is differentiated from it by the susceptibility of Marquis and intermediate reaction of Canus.

1918 but surveys made in 1927 and 1928 showed that T. foetida was present in all the principal wheat-growing districts of this State. However, T. caries was still the predominant species. In 1929 and 1930 Flor (2) made collections of bunt from 182 fields in the principal wheat-producing areas of Oregon, Washington, and northern Idaho, and found T. caries in every collection and T. foetida in 60 per cent of the collections.

The present report is based on studies with 369 collections³ of *Tilletia* caries and *T. foetida* made from 1932 to 1942. As shown in tables 3 and 4, they were obtained from 35 states in the United States, 6 states in Mexico.

³ A few field collections were mixtures of the two species or more than one race. When isolated each was given a separate collection number.

and 2 provinces in Canada. Of the total number of collections 62 were T. caries, obtained from 15 states and provinces, and 307 were T. foetida, obtained from 41 states and provinces. Thus, T. foetida has a much wider distribution than T. caries, the former being found in all except one of the States and Provinces from which collections were obtained and the latter in less than a third of the collections. The increase of T. foetida in the Northwest noted in previous surveys is corroborated by the results of the present survey. Of 94 collections made in the far western states, including

958

TABLE 3.—Number and distribution of physiologic races of Tilletia caries in 62 collections from North America.

					Nur	nber	of	colle	ctio	ns o	f ra	ces					suc	H
Source of collections	T-1	T-2	T-3	T-4	T-5	JC	T-7	T-8	6-L	T-10	T-11	T-12	T-13	T-14	T-15	T-16	Total collections	Number of races
United States															-			-
Arizona California Georgia	2 15	 1		1		,,,,,,	,,,,,,								,		$\begin{array}{c} 2\\16\\1\end{array}$	$egin{array}{c} 1 \ 2 \ 1 \end{array}$
Idaho Minnesota	1	1	1		*****	1	7	2						3	1		6 1 6	4 1 5 2
Montana New York North				1	1					,							2	-
Dakota Oregon South	*****		1	******	*****	ï	;	1	1			1			, , ,		4 5	1 5
Dakota Washington West Vir-	ī	1				1	3	1	1	1	1		1				1 10	1 8
ginia Wyoming		2		******				1		*****					•••••	1	$\frac{2}{2}$	2 1
Canada																		
Manitoba			•••••	******	,	1		,								•••••	1	1
Mexico Sonora																		
Totals	$\frac{2}{21}$	9	3	2	1	5	 4	 5	2	, T	 2	٦	1	3			$\frac{3}{62}$	2
Per cent	33.9		5 4.	_	2 1.	-		5 8.		2 1	-	_			.8 1	.6 1.		

Colorado and Wyoming, 41 were *T. caries* and 53 were *T. foetida*. East of the Rocky Mountains, *T. foetida* continued to predominate as shown by the fact that, of 238 collections, 221 were *T. foetida* and only 17 were *T. caries*. It seems evident that *T. foetida* has spread to new regions more readily than *T. caries*. The explanation for this apparent wider adaptation of *T. foetida* is not indicated by the data at hand.

Races of Tilletia caries

The distribution of the races of *Tilletia caries* is shown in table 3. The 16 races of this species, identified in 62 collections, were distributed in 13 states in the United States, one state in Mexico, and one province in Canada.

Thus, on the average, a different race was identified for approximately every four collections of the species. This high ratio is partly explained by the fact that several races that were previously identified by other investigators

TABLE 4.—Number and distribution of physiologic races of Tilletia foetida in 307 collections from North America

[-]																(1)
Τ	12-2	L-3	15-4	L-5	17-6	I7	I8	6-T	L-10	I-11	L-12	L-13	L-14	L-15	Total collections	Number
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2	4	2					1			3				*****	12	5
		1	1	******			-5								7	. 3
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		3													12	4
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															3	1
		6													10	3
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8			1							3	1				13	- 4
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were included in the collections. Therefore, strictly speaking, all of the so-called collections were not actually field collections.

The number of collections of each race ranged from 1 to 21. Races T-1 and T-2 were the most prevalent, constituting 33.9 and 14.5 per cent, respectively, of the collections of Tilletia caries. The former, identified only from collections in four western states and Sonora, Mexico, greatly predominated in collections from California where 15 of the 16 collections of this species were T-1. That it was collected only once in Idaho and once in Washington probably is due to the fact that these states had been thoroughly surveyed by earlier workers and in the present studies emphasis was placed on collections from varieties that are resistant to T-1. Race T-2 was identified in collections from five states over the hard red spring and the hard and soft red winter wheat areas but it is apparently commonest in the durum section of the hard red spring wheat area. As shown in table 1, this race is relatively innocuous on all but one of the winter wheat differential varieties; however, it is highly virulent on Mindum and certain other durums, thus complicating the problem of breeding for bunt resistance in this class of wheats. Races T-6 and T-8 were found in 5 collections each, the former being obtained from 5 areas and those of the latter from 4 areas. Both of these races are important factors in the winter and spring wheat improvement program. Six races, namely, T-5, T-10, T-12, T-13, T-15, and T-16, were each found in only one collection. However, this does not greatly minimize their importance, because of the fact that bunt is accumulative. Consequently, in a wheat improvement program, all races should be considered, even though for the time being some of them appear to be of uncommon occurrence.

Classification of the dwarf bunt fungus in relation to the races of *Tilletia caries* that cause ordinary bunt is being held in abeyance until methods have been devised whereby comparable pathogenicity tests may be made. However, because of the importance of dwarf bunt in certain wheat-growing areas, information on its occurrence and distribution is needed. Dwarf bunt first attracted attention as a regional problem in wheat production in 1931. At that time it was observed in devastating proportions over a fairly wide area in southeastern Idaho and northern Utah. In the same year it was observed on the High Prairie area of Klickitat County in south-central Washington by Roderick Sprague and in the Gallatin Valley near Bozeman, Montana, by P. A. Young and H. E. Morris. Since then, other reports of dwarf bunt have shown that it now occurs over a much wider area in these states.

The most extensive area of dwarf bunt infestation apparently is in Idaho where it occurs (1) throughout the dry-land wheat area of the southeast portion; (2) in the prairie region around Winchester and Nesperce; (3) on the ridges east of Troy; (4) in the Palouse near Genessee; and (5) in the semi-timbered section around Worley and Plummer. Thus, in Idaho, the

⁴ Observations reported by representatives of the Division of Cereal Crops and Diseases and cooperating agricultural experiment stations in Idaho and Utah.

infested areas are distributed from the southern border to the panhandle. In Washington, in addition to the infested area on High Prairie (Klickitat County), which extends eastward to the Goldendale area, dwarf bunt has been observed near Waterville (Douglas County), near Wilbur (Lincoln County), and near Spangle (Spokane County). The occurrence of dwarf bunt in Wyoming, adjacent to the southeastern border of Idaho, has been reported by Blodgett (1).

Dwarf bunt infestation near Bozeman, Mont., was observed as early as 1931. Recently it has been reported by R. H. Bamberg and H. E. Morris to occur in destructive proportions in the northwestern part of the State extending from 20 miles south of Flathead Lake to 10 miles north of Kalispell. In Utah, the first area of infestation observed was in the Cache Valley near Logan, Cache County, and in Box Elder County by D. C. Tingey and R. W. Woodward. During the past 10 years it has spread and is now established over the entire dry farm areas of the northern part of the State. It also is locally distributed in Salt Lake County and as far south as the eastern end of Juab County near Nephi.

Dwarf bunt has been found also in Colorado⁵ and New York⁶ as indicated by reports verified by specimens examined by the writers.

Races of Tilletia foetida

A total of 307 collections, or 83.2 per cent of those studied, proved to be Tilletia foetida. The distribution of the 15 races identified is shown in table 4. They were distributed in 34 states in the United States, six states in Mexico, and one province in Canada. Thus, on the average a different race was identified for approximately every 20 collections. The number of collections of each race ranged from 1 to 78. Races L-1, L-2, L-3, and L-4 predominated with respect to number of times collected and range of distribution. Of these, L-1 was most prevalent and widely distributed, having been collected 78 times in a total of 25 states, including those of Mexico. Sixty-one collections from 13 states and provinces yielded L-2, and 60 collections from 18 states, in the United States only, yielded L-3. Next in relative prevalence was L-4, which was found in 37 collections from 18 states, in the United States only. L-2 and L-3, which have a limited range in pathogenicity on the winter wheat differential varieties, were the commonest races of T. foetida collected in the hard red spring wheat area. One of the five new races of T. foetida, L-11, reported for the first time in this paper, was widely distributed, having been collected in seven eastern and midwestern states and in California.

The twenty-nine collections of *Tilletia foetida* from 6 states in Mexico yielded 6 races, three of which are new, namely, L-13, L-14, and L-15. Of these three, only L-15 has been found in the United States. Race L-2, which is among the four most common races in the United States, also was the most

6 Observed by B. B. Bayles who furnished specimens for laboratory study.

⁵ Report by E. W. Bodine and L. W. Durrell in Plant Disease Reporter 25: 485, 1941; and in correspondence with E. F. Darley.

TABLE 5.—Percentage of bunt produced on certain varieties and strains of Triticum aestirum L., T. durum Desf., and T. timopheevi Zhuk. by physiologic races of Tilletia caries and T. foetida

										Д	erce	Percentage of	ge o		lms	infe	culms infected	þ.	4.					-				
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	Species, class, variety, or hybrid		Soft red winter Fultz × Hungarian Hope × Hussar Hask × Hohenheimer Hus × Red Wave	White spring Baart 38 Federation Federation 41 Federation 41 Federation 41 Federation 41	Hope x Federation Marflum Marduris-Flor x Fed. Onas 41	White Fell. 30 White wher Brevon Hymar Requa Rex Turk, x Florence Wooth of Sol 6	T. durum Mindum Cariton Stewart	T. timopheevi x T. aestivum Sel. H143-1-1-8-110 Sel. H143-1-1-13-8 Sel. H143-1-1-3-13 Sel. H143-1-1-1-1 Sel. H143-1-1-1-1

common one in Mexico. The new races L-14 and L-15 have wider host ranges than L-2 and were found 7 and 4 times, respectively, in Mexico. It is also noteworthy that L-8, which, in the United States, was found only among the winter wheats of the western region, was collected once as far south as Puebla.

VARIETAL RESISTANCE

The varietal tests with different races of bunt reported in this paper were made to obtain information that would assist plant breeders in selecting parent material to use in the development of bunt-resistant varieties. The varieties tested include a number of commercially grown spring and winter wheats, some hybrid selections that in preliminary experiments had resistance to at least some of the known races, and a few varieties now being used as breeding material for rust resistance. The technique employed in preparing, inoculating, and planting the seed was the same as that previously described for experiments made in determining physiologic races. The spring wheats were tested at Aberdeen, Idaho, and the winter wheats at Pullman, Wash. All of the tests were not made the same year and for this reason the reactions for all varieties are not strictly comparable. The data presented, however, were obtained in years when environmental conditions favored high infection as evidenced by the fact that each year the winter wheat check variety, Hybrid 128 (C.I. 4512), had on the average more than 75 per cent bunt, and the spring wheat check, Ulka (C.I. 11478), from 85 to 99 per cent for all races to which it is known to be susceptible. Furthermore, there was excellent differentiation between races on the standard differential varieties, which were included each year in the same plots for comparison. The smut percentages are based on total number of culms, averaging 300 per 5-foot row for the spring varieties and 250 per 6-foot row for the winter ones. Fractional percentages of 0.5 or more were recorded as 1 per cent, those below this fraction as a "trace" (T).

With a few exceptions, the results of the varietal tests with physiologic races are summarized in table 5. The data on the following 13 varieties and selections are omitted because these varieties were all either intermediate or susceptible in reaction to each of the races, and, therefore, are of no value as parent material in breeding for resistance to bunt. These varieties are Cadet (C.I. 12053), Frondoso (C.I. 12078), Frontiera (C.I. 12019), Kenya (C.I. 12332), McMurachy (C.I. 11876), Red Egyptian (C.I. 12345), Akron No. 7 (C.I. 11660), Tenmarq (C.I. 6936), Carlson's Fife (C.I. 11922), Elgin (C.I. 11755), Hindi (C.I. 8454), Jenkin (C.I. 5175), and No Name (C.I. 12301).

It is evident from the data presented in table 5 that there are a number of wheats that possess factors for resistance to all of the races of both species of the bunt fungi insofar as the races have been definitely delimited. There are five hard red spring wheats, four hard red winter wheats, and *Triticum*

⁷ C.I. refers to accession number of the Division of Cereal Crops and Diseases.

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timopheevi that are generally resistant. In the other groups, no generally resistant varieties have been found.

The five generally resistant hard red spring wheats are (1) Hope, (2) Komar × Hussar selection, (3) Regent × Pilot selection, (4) Reliance-1018 × Mercury selection, and (5) Renown. Except for Komar × Hussar selection, the resistance in these wheats, when sown in the spring, is apparently conditioned by the Hope factor. It is known, however, that certain spring wheat varieties are more resistant when planted in the spring than in the fall (3, 9, 11, 12). Hope is in this group and although it and the hybrids that have this variety as one of the parents were highly resistant when planted in the spring at Aberdeen, Idaho, it is quite possible they will be susceptible if planted in the fall. The high degree of resistance of the Komar × Hussar selection may possibly be due to transgressive segregation, as neither of the parents, which have been tested extensively in Uniform Bunt Nursery experiments with composite inoculum of some of the races, have had the resistance shown by the hybrid in these tests.

The four generally resistant hard red winter wheats are selections of (1) Oro × Turkey-Florence, (2) Rex × Oro, (3) Rex × Rio, and (4) Rio × Rex. It is also noteworthy that a selection of H-44 × Minturki (C.I. 12414) was resistant to all races except T-15 and T-16, to which it was intermediate in reaction. In general, however, it appears that both Hope and H-44 are less satisfactory as sources of bunt resistance for breeding winter wheats than they are for spring wheats. Thus in preliminary experiments at Pullman, Wash., in 1942, selections from 16 hybrid lines involving Hope with the winter wheats Cheyenne, Kanred, Mediterranean, Kawvale, Red Rock, and Fultz, and 8 lines of H-44 with Minturki were tested with a composite of races L-2, L-8, and T-8. One selection from the Hope hybrids and three selections involving H-44 were intermediate in reaction while the other 13 were completely susceptible.

Of the white spring wheats, a selection of Hope × Federation (C.I. 12423) showed some degree of resistance to all races and Orfed was highly resistant to all races except T-16 and L-8. Of the white winter wheats, Washington Selection 6, was intermediate in reaction to T-16 and L-8 and resistant to all the other races.

Unfortunately the new races from Mexico were not used in the tests. However, the resistance of Ridit and Oro to these races (Table 1) indicates to some extent how certain other varieties might react, and insures the availability of a resistance factor for developing new varieties resistant to these races should they become a menace to wheat production in the United States.

Triticum timopheevi (C.I. 11802) was the only wheat tested that was free from infection by all races. Shands (10) has succeeded in hybridizing T. timopheevi and a common wheat T. aestivum and five of his selections were included in these tests. The high degree of general resistance of the T. timopheevi parent, however, was not recovered in the hybrids. Each selection was resistant to some races, although the different selections were not always similarly resistant, intermediate, or susceptible to the different races.

Owing to the nature of the dwarf bunt fungus, pathogenicity tests comparable with those of the other races could not be made. There is evidence. however (5, 8, 14), that the so-called Hussar, Ridit, and Martin factors effectively control resistance to this bunt. Several hybrid strains deriving their resistance to dwarf bunt from one or more of these wheats were used in the present experiments, namely, Relief (Hussar × Turkey, Utah No. 26), Cache (Ridit × Utah Kanred), Hymar (Hybrid 128 × Martin) and Wasatch (Ridit × Relief). As would be expected from their parentage, these hybrids were resistant to a number of races but developed considerable bunt when inoculated with those races to which Hussar, Ridit, and Martin are susceptible. Oro × Turkey-Florence selection, on the other hand, is not only highly resistant to all of the races of ordinary bunt, but also has been resistant to dwarf bunt in several tests (5). However, the results have been somewhat conflicting inasmuch as this variety developed 22 per cent of dwarf bunt in one of the tests at Logan, Utah. Actually it probably has the same resistance to this bunt as Ridit and Cache, which is not adequate in areas of severe infestation. Theoretically, the variety combinations which offer the best possibility for producing resistance to all races, including dwarf bunt, are those that bring together the Oro and Martin types of resistance, which are carried by Rio and Rex, respectively. Data in table 5 on three hybrids of this type, namely, Rex × Oro, Rio × Rex, and Rex × Rio, show that they are highly resistant to all the races of ordinary bunt. Preliminary results' indicate that Rex × Oro and Rio × Rex are also highly resistant to dwarf bunt but no dwarf bunt tests have been made with Rex × Rio.

DISCUSSION AND CONCLUSIONS

Several factors probably operate to determine the distribution and relative prevalence of races of Tilletia caries and T. foetida. Among them, no doubt, are the direct effects of environment on the pathogen, on the host, and on the interrelation of the two as well as the distribution of wheat varieties. Regarding the influence of environment on the host, there is evidence that some varieties vary greatly in their reaction to certain races under different environmental conditions (7, 9). Obviously this factor might effect a build-up of inoculum of certain races in some localities. However, this probably is a minor factor, since the response of other varieties to the same races appeared to be unaffected by change in environment. Probably the distribution of specific wheat varieties is the most important factor governing the distribution and relative prevalence of the different races. Flor (2) found, in his survey of races of Tilletia foetida and T. caries in the Pacific Northwest, that the less virulent races of both species predominated on the old commercially grown varieties. While the more virulent races were found occasionally on the old varieties, they were more common in the districts where such varieties as Albit and Ridit were grown. From the results of the present survey, a similar condition appears to prevail in all wheat-growing

8 Unpublished data from the Cooperative Uniform Dwarf Bunt Nurseries of 1944.

areas of the United States. Thus, for example, although L-8 was collected in California and in Puebla, Mexico, on varieties of wheat that are susceptible to all of the known races, it has become increasingly prevalent in certain areas of the Northwest where Oro and Yogo are widely grown. Similarly, race T-2 was found in such widely separated areas as Wyoming, North Dakota, and Georgia, but its importance coincides with the distribution of the durum wheats in the hard red spring wheat area. More recent changes in the varieties grown in certain other wheat-growing areas may afford opportunity for further study of this factor as it affects the distribution of For example, in the Southwest, Baart and White Federation, which are susceptible to the predominating races (L-1, L-2, L-3, and T-1) in that area, are being replaced by Baart 38 and White Federation 38. These new varieties should eliminate these races but, on the other hand, they may favor an increase in prevalence of the so-called Martin races to which they are susceptible. Likewise, it will be of interest to note whether the distribution of the new Turkey type wheats, Nebred and Comanche, will effect a build-up of race L-8 in the Nebraska and Kansas hard red winter wheat area.

It is evident from the study of the bunt reaction of different varieties (Table 5) that there is ample bunt-resistant breeding material. At the present time the Hope factor seems adequate to control the known races of bunt infecting spring wheats while combinations of factors from Oro with Martin or Ridit control the races that infect the winter wheats. For example, factors governing the so-called Martin reaction in the variety Rex when combined with those governing the Oro type reaction of the Rio parent gave a hybrid resistant to all races. Likewise, combining the Oro factors with those of Turkey-Florence, which supplied the so-called Ridit reaction, gave equally satisfactory results. Hybrids between Martin and Ridit were not available for the present studies. It should be possible, however, to derive from such crosses lines that would be resistant to all races since none of those infecting Martin infect Ridit, and vice versa.

The problem of breeding bunt-resistant varieties for certain sections of Utah, Idaho, Montana, and Washington is complicated by the occurrence of the dwarf bunt. The commercially grown varieties, Relief, Cache, and Wasatch, are resistant to dwarf bunt but are susceptible to certain races of both Tilletia foetida and T. caries that occur in those areas. There remains, therefore, the problem of combining the factors governing resistance to dwarf bunt with those governing resistance to races of both species of the ordinary bunt. Variety combinations that, theoretically, should produce this type of resistance would include as one parent, Rex, Hymar, Relief, or Wasatch, and as the other parent, Oro, Rio, Yogo, or Nebred.

SUMMARY

Seven new races of *Tilletia caries* and *T. foetida* are described, making the total number of known races 31, of which 16 are of the former species and 15 of the latter. Three of the new races of *T. foetida* were identified from collections made in Mexico.

Evidence corroborating results of previous surveys was obtained relative to the general distribution of the two species of the bunt fungi. *Tilletia foetida* is now the predominant species throughout the United States but *T. caries* is found over a considerable area. It is common in the durum section of the Upper Mississippi Valley and greatly increases in relative prevalence toward the intermountain area of the Northwest where it formerly was the predominant species.

The distribution of 16 races identified from 62 collections of *Tilletia* caries and 15 races from 307 collections of *T. foetida* are shown. Several of the races are widely distributed while others are more limited in their distribution. It seems evident that the distribution of specific wheat varieties is the most important factor governing the prevalence of specific races within a given area. Thus, for example, races T-11 and L-8 have assumed increasingly greater importance in the areas where Ridit and Oro, respectively, have replaced the old commercial varieties that had served as hosts of the more common races.

A number of varieties and selections were resistant to all of the races of ordinary bunt that were tested. It seems evident that thus far the Hope factor adequately controls these races in the spring wheats while lines derived by combining the so-called Oro and Martin factors, as in ${\rm Rex} \times {\rm Oro}$ and ${\rm Rio} \times {\rm Rex}$, supply the necessary resistance for the winter types. Theoretically, selections from crosses between Martin and Ridit should likewise possess the desired resistance.

Dwarf bunt is a real menace to wheat production, particularly in certain sections of Utah, Idaho, and Montana. It is increasing in importance in the State of Washington and has been found in Wyoming, Colorado, and New York. The varieties, Relief, Cache, and Wasatch, although resistant to dwarf bunt are susceptible to a few races of the ordinary bunt fungi, hence there remains the problem of combining the factors that will supply resistance to both types.

DIVISION OF CEREAL CROPS AND DISEASES, U. S. DEPARTMENT OF AGRICULTURE.

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NEW PHYSIOLOGIC RACES OF USTILAGO HORDEI1

V. F. TAPKE2

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INTRODUCTION

The importance of physiologic races of covered smut (*Ustilago hordei* (Pers.) Lagerh.) in the breeding and distribution of resistant varieties of barley in the United States is well recognized. In a previous paper (10) the writer reported the isolation of 8 clear-cut races among 200 covered smut collections from 26 States. Since the publication of that paper, 244 additional collections from barley-growing States have been studied. Five new races have been identified. The reactions of these races on the differential varieties and the distribution of races in the States from which collections were obtained are presented herewith.

PREVIOUS INVESTIGATIONS

The writer's earlier report (10) reviewed the previous studies up to 1937. Since then, Allison (1) in a one-year test of 28 collections on 11 varieties of barley found evidence of pathogenic differences in 27 of the lot but hesitated to consider all of them as distinct races. Semeniuk (9) studied 12 Canadian collections of *Ustilago hordei* and obtained evidence of 4 races, but in a later test the distinctions between 3 of the races had practically disappeared.

MATERIALS AND METHODS

The tests were conducted under field conditions at Ithaca, N. Y., 1939 to 1942, inclusive. The technique of seed inoculation, seeding, and other phases of experimentation followed that described in the report of the earlier studies at Ithaca (10). The pure-line differential varieties likewise were the same except that the black seeded variety Gatami (C.I. 575) was replaced by Himalaya (C.I. 1312). Smutted heads, especially the partly smutted ones, are difficult to detect in Gatami. A total of the 244 collections from 27 States were studied.

The seed used in each year of the test came from the previous season's crop produced under irrigation at Aberdeen, Idaho. This practice also was followed in the writer's previously reported tests (10) although a statement to this effect was not included. Judged by the results of Holton and Heald (5) with bunt in wheat and of Tervet (14, 15, 16) with loose and covered smuts in oats, the use of barley seed lots produced at one place in the same

¹ Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the New York (Cornell) Agricultural Experiment Station.

² Pathologist, Division of Cereal Crops and Diseases. The writer is indebted to Dr. H. H. Love and Mr. W. T. Craig, Department of Plant Breeding at Cornell University, for supervision of the field plantings at Ithaca, N. Y., and for other helpful assistance.

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season probably is important in physiologic race studies of the seedling infecting barley smuts *Ustilago hordei* and *U. nigra*. These investigators found that the various lots of a variety grown in different localities in the same year often show wide differences in susceptibility when artificially inoculated. Tervet (14, 15, 16) also obtained similar results with the various lots of varieties grown in the same locality in different years.

EXPERIMENTAL RESULTS

New Physiologic Races

Five new races (numbered 9 to 13) have been identified by their reactions on the 8 differential varieties during the 4 years 1939–1942. The

TABLE 1.—Average percentages of smutted heads in 8 varieties of spring barley inoculated with 13 physiologic races of Ustilago horder and grown at Ithaca, New York, 1934-42

			Av	erage perc	entages	of smutte	d heads	in	
Race No.	Number of years tested	Excelsior C.I. 1248	Hannchen C.I. 531	Himalaya C.I. 1312	Lion C.I. 923	Nepal C.I. 595	Odessa C.I. 934	Pannier C.I. 1330	Trebi C.I. 936
1 2 3 4 5 6 7 8 9 10 11 12 13	7 7 7 7 7 6 6 4 4 4 4	0.0 0.6 24.0 0.4 0.0 0.0 1.0 0.0 13.0 0.0 0.0 Trace 23.0	20.0 0.3 0.0 8.0 0.0 28.0 0.0 10.0 13.0 27.0 10.0 16.0	0.0 0.0 0.0 12.0 0.0 0.3 0.0 0.0 0.0 10.0 11.0 12.0 3.0	0.0 14.0 12.0 0.0 16.0 22.0 0.0 6.0 11.0 24.0 0.0 14.0	0.0 50.0 50.0 35.0 0.0 0.0 33.0 0.2 40.0 43.0 13.0 50.0 37.0	39.0 34.0 42.0 30.0 38.0 45.0 34.0 37.0 32.0 26.0 39.0 39.0 37.0	0.0 0.0 0.6 13.0 0.0 0.3 0.0 0.3 14.0 0.0 0.8	6.0 0.6 0.1 13.0 24.0 40.0 5.0 0.0 11.0 43.0 0.5 18.0

average percentages of infection obtained on the varieties with these races are in table 1. Included in the table are the averages of the previously isolated races 1 to 8 during the 1939–1942 period and the earlier years in which they were carried (10).

Each of the 5 new races (9, 10, 11, 12, and 13), as shown in table 1, had a distinctive pathogenic action.

Most of the collections of covered smut produced clear-cut reactions when first tested and were evidently composed of but a single race. Not infrequently, however, the reaction of the differential hosts indicated that a small admixture of one or more other races might be present. With the aid of screening hosts, 2 races were isolated from some collections and from an Arizona collection 3 races were obtained including the first isolation of race 13. In barley covered smut, at least thus far, there has been no evidence such as reported in oat smuts (8) and wheat bunt (2, 6) of spontaneous

origin of new races from previously stable races through mutation or segregation of factors for pathogenicity in heterozygous spores.

Minor differences in size and color of chlamydospores, smoothness of spore walls, compactness of the smutted heads and of the spore masses, degree of destruction of awns, and degree of exsertion of the smutted heads from the boot are not infrequently associated with differences in the pathogenicity of physiologic races of *Ustilago hordei*. The heads smutted by race 12 for example, are less compact and more spongy than those smutted by other races; they usually break out of the boot, as shown in figure 1; and the spore walls of the chlamydospores are slightly echinulate. Other investigators (4, 6) have previously reported similar variations in physiologic races of other cereal smuts. The magnitude of these differences is, in general, insufficient for definite identifications.

From the behavior of the 8 differential hosts, it is evident that even from this limited selection of varieties the control of covered smut through breeding for resistance should not be too difficult. The variety, Pannier (C.I. 1330), for example, was appreciably susceptible only to the infrequently collected covered smut races 4 and 10 and to date it has also proved immune from all 7 of the known physiologic races of the black or shallowborne barley loose smut caused by *Ustilago nigra* (11, 12).

Finally, in connection with the data of table 1, it is interesting to recall that getting high percentages of covered smut in susceptible barleys under field conditions through artificial inoculation of the seed was formerly a difficult problem. The consistently satisfactory infections obtained through use of the spore-suspension method of seed inoculation (13) during the 7 years of these tests afford ample proof of the effectiveness of this method.

Stability of Races

In studies of differential spring wheat varieties that were inoculated with different physiologic races of the bunt fungi (Tilletia levis and T. tritici) and grown at different experiment stations, Rodenhiser and Holton (7) found that the susceptibility of a certain variety to a certain race in a particular year, for example, might be uniform at the different stations but the susceptibility of some other variety to this race might be relatively high at some stations and low at others. Similar results were obtained in the reactions at individual stations in the course of several years. Rodenhiser and Holton were inclined to the view that under the different environmental conditions the expression of genetic factors for protoplasmic resistance in certain varieties to certain races may be modified. During the course of the present study with barley covered smut at Ithaca, New York, a wide variety of climatic conditions was encountered but the relative susceptibilities of the differential varieties to each of the 13 races appear to have been adequately maintained. With race 6, for example, as shown in table 2, the varieties Excelsior, Himalaya, Nepal, and Pannier maintained their status of immunity or near immunity throughout the test. The percentages of smut in the susceptible Hannchen, Lion, Odessa, and Trebi varied widely under the different yearly conditions but each variety still

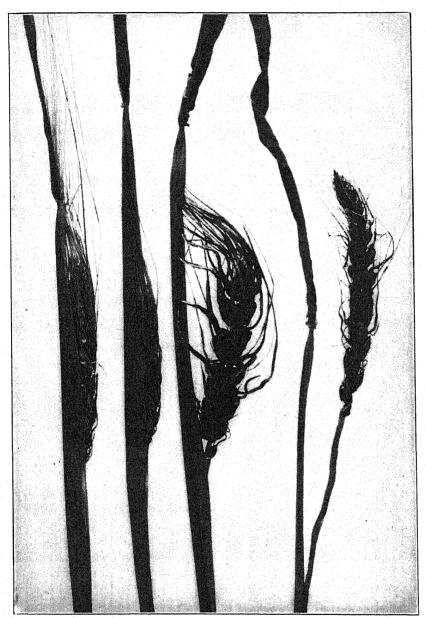


Fig. 1. Heads of Odessa (C.I. 934) barley smutted by covered smut; the two heads at left by race 7, the two at right by race 12, showing differences in morphologic expression.

maintained its relative degree of susceptibility. With few exceptions in the 7-year test, Hannchen was more susceptible to race 6 than Lion; Trebi was

more susceptible than Hannchen; and Odessa was more susceptible than Trebi. Similar general consistencies in reaction occurred with the other races.

Occurrence and Distribution of Races

Table 3 shows the frequency of occurrence of races and their distribution by States. The data include 244 collections of the present study and 200 collections of the similar, previous study (10). This gives a comprehensive picture of the frequency of occurrence and distribution by geographic divisions and States of 444 collections of *Ustilago hordei* from 33 barley-producing States.

Races 1, 5, and 6, comprised 86.5 per cent of the total collections. Race 6 is by far the most generally important. It occurred in 61.3 per cent of the 444 collections and is widely distributed. Race 5 appears largely confined to the far western States but it is very important in California and Washington where it comprised 63 of the 74 collections from these

TABLE 2.—Percentages of smutted heads produced by physiologic race 6 in 8 differential varieties of barley over a period of 7 years at Ithaca, N. Y.

Year			Percent	ages of	smutted her	ids in		
of test	Excelsion	Hannehen	Himalaya	Lion	Nepa1	Odessa	Pannier	Trebi
1934	0.0	30.0		26.0	0.0	53.0	0.0	46.0
1935	0.0	17.0		20.0	0.0	34.0	0.0	30.0
1936	0.0	32.0		19.0	0.0	50.0	0.0	31.0
1939	0.0	42.0	0.0	28.0	0.0	51.0	0.0	60.0
1940	0.0	23.0	1.0	16.0	0.0	46.0	0.0	38.0
1941	0.0	21.0	0.0	15.0	0.0	31.0	0.0	31.0
1942	0.0	33.0	0.0	27.0	0.0	51.0	0.0	42.0

States. Race 1 has a wide distribution and was frequently collected in the winter-barley area as defined by Harlan and Wiebe (3). There seems to be some relation between the regions chiefly inhabited by races 1, 5, and 6, and the barley types grown there. Race 1 predominates in the winter barley region, race 5 in the region of Coast type barleys, and race 6 in the region of the Manchuria-Oderbrucker type. Race 8 was found more frequently in Kansas than elsewhere. The remaining 9 races were infrequently collected and together comprised approximately 9 per cent of all collections. It is evident that, at least at present, certain races of the barley covered smut fungus are highly important in certain areas while others are relatively unimportant there. This information should facilitate breeding for covered smut resistance.

Finally, as shown in table 3, from 1 to as many as 6 different races were found in individual States.

SUMMARY

Thirteen physiologic races of *Ustilago hordei* have been isolated, 5 in the present study of 244 collections and 8 in a similar previous study of 200 collections.

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The combined results, involving 444 collections from 33 States, show that the widespread race 6 comprised 61.3 per cent of the total collections. The 3 most prevalent races, 1, 5, and 6, comprised 86.5 per cent of the total. In the winter barley area race 1 appears to be somewhat more prevalent than

TABLE 3.—Frequency of occurrence and distribution by Grouped States of physiologic races of Ustilago hordei in 444 collections from 33 States, 1934-1942

Stoton				Nt	ımber	of col	lecti	ons o	f rac	e				Total
States	1	2	3	4	5	6	7	8	9	10	11	12	13	collec- tions
North Atlantic														
Maine						1	******			*****	******			1
New York						10								10
Pennsylvania	2		******			6								8
North Central														
Illinois					1	4	3	- 1						.9
Iowa	2		2			37					1	1		43
Kansas	-		1		1	7		8	******					17
Michigan						5		-						5
~	2				******	41			1	•••••	4			48
	7		1	******	•••••				_		_			13
Missouri	-			*****	• •••••	1		4				******	*****	
Nebraska		•••••			*****	11	• • • • • • • • • • • • • • • • • • • •	******		*****	*****			11
North Dakota	1	*****	•	1	******	30	*****		. 1					33
South Dakota	1		*****			29	*****	1	4	. 1	3	*****	*****	. 39
Wisconsin	1				1	36	•••••						*****	38
South Atlantic														
Georgia	3	******			*****									3
Maryland	1				*****		*****	*****						1
North Carolina	2	******				1								3
South Carolina	1		•••••		•••••			•••••	1	*****				2
	10			*****	******	1	*****	3		******	******		******	15
Virginia		******	1	*****	******				•••••	******		*****	•••••	
West Virginia	•••••			*****		1	*****	1	******	******	*****		*****	2
South Central														
Louisiana						1		*****						1
Mississippi	******	******		*****	******				1		******			1
Oklahoma	4					- 5		2	*****		******		******	11
Tennessee	1			*****	•••••	2							******	. 3
Texas	1				******	2		******	******					3
Far Western		*****	******		*****	- : -								Ŭ
Arizona				1	2	2							1	6
~	1	*****	1		42	1	******	•••••	******		******			45
~ - >						i	. ******	******	******	*****	******		******	1
T 1 .	******	2					******	•••••			*****	•••••		20
Idaho	******	Z	1		2	14	*****			1	*****	•••••		
Montana			*****	. 2		2		******		******		******	••••	4
Oregon		1	*****	1	1			******	•••••		******			3
Utah		*****			1	14			*****			*****		15
Washington			1	1	21	6				******		•••••		29
Wyoming			••••		•••••	1	•	,	• • • • • • • • • • • • • • • • • • • •	*****	•••••			1
Total collections	40	3	8	6	72	272	3	20	8	2	8	1	٦	444
of each race	40	ฮ	ð	v	12	212	3	20	. 0	2	ŏ	1	1	444
Percentage of all collections	9.0	0.7	1.8	1.4	16.2	61.3	0.7	4.5	1.8	0.5	1.8	0.2	0.2	

race 6. In California and Washington, home of the Coast type barleys, race 5 was by far the most prevalent, comprising 85.1 per cent of the 74 collections from these States. Elsewhere throughout the country where, in general, the Manchuria-Oderbrucker barley types prevail, race 6 usually surpassed all others.

From 1 to 6 races were found in individual States.

During the 4 to 7 seasons that each of the 13 races was tested at Ithaca, N. Y., the 8 differential varieties of barley maintained their relative susceptibilities to each of the races although the climatic conditions within and between seasons often varied widely.

Not infrequently associated with different physiologic races of Ustilago hordei are minor differences in size and color of chlamydospores, smoothness of spore walls, compactness of the smutted heads and spore masses, degree of destruction of awns, and degree of exsertion of the smutted heads from the boot.

Among the differential varieties, Pannier (C.I. 1330) showed promise as foundation stock in breeding for smut resistance.

Consistently satisfactory infections throughout the 7 years of these tests through use of the spore suspension-method of seed inoculation afford proof of the effectiveness of this method.

DIVISION OF CEREAL CROPS AND DISEASES.

U. S. DEPARTMENT OF AGRICULTURE,

AND

CORNELL AGRICULTURAL EXPERIMENT STATION, ITHACA, NEW YORK.

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A NEW SPECIES OF COLLETOTRICHUM ON VETCH1

J. L. WEIMER

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INTRODUCTION

During the past several years, routine observations have been made on the diseases of vetches (Vicia spp.) among which was one that in certain characters resembled the diseases caused by Kabatiella nigricans (Atk. and Edg.) Karak., Colletotrichum viciae Dearn. and Overh., and Ascochyta spp. The diseases caused by K. nigricans and Ascochyta spp. often were present along with the anthracnose under discussion in this paper and it now appears that they were sometimes confused with it. It was finally decided that a disease complex existed that needed clarification and an investigation was initiated, the results of which form the basis of this paper.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Little information regarding the distribution and severity of the anthracnose reported herein is available at present, other than the limited observations made by the writer near Experiment, Georgia, and a collection made by Dr. C. L. Lefebvre at Quincy, Florida. Dr. B. B. Higgins has observed an anthracnose on vetches at Experiment, Georgia, for a number of years but has made no study of it.

Considerable defoliation of smooth and hairy vetches is caused by the fungus during rainy seasons. Stems are blackened, and some stems are killed; but it is only in wet seasons when the crop is grown continuously on the same land that the disease becomes sufficiently abundant to damage commercial plantings.

SYMPTOMATOLOGY

On the leaves the lesions characterizing this disease are somewhat circular, but they may be elliptical or angular when delimited by veins, the margins of the leaflets, or an adjacent spot (Fig. 1). Although lesions vary in size, they are commonly from 1 to 2 mm. in diameter. There may be one or many lesions on a leaflet: sometimes the points of infection are so numerous that adjacent lesions coalesce and a large portion or even the entire leaflet may be involved (Fig. 2, A). In early stages the invaded tissues are light green, sometimes whitish or yellow-olive. Even at this early stage the lesion has usually reached its maximum size. The color gradually fades until it becomes light brown, gray, or nearly white. The central area, which makes up half or more of the diameter of the spot, is surrounded by

¹ Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Georgia Agricultural Experiment Station. Paper number 138, Journal Series, Georgia Agricultural Experiment Station.

² Ridgway, Robert. Color standards and color nomenclature. 43 pp., 53 color plates. (Washington, 1912.)

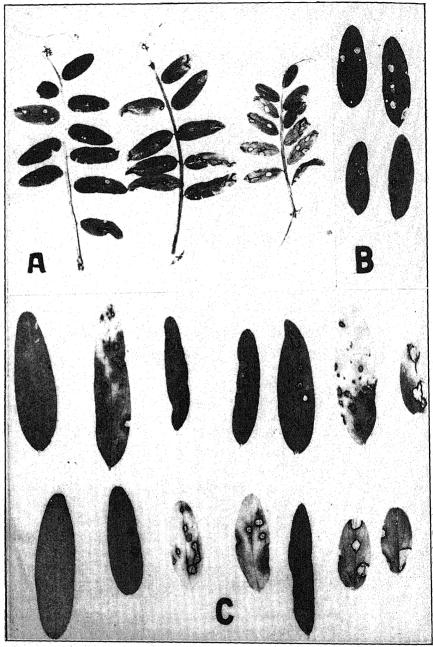


Fig. 1. Anthracnose leaf spot of vetch. A. Smooth vetch leaves showing shape and distribution of the spots on the leaflets. The red border surrounds the light center of the lesion or is adjacent to healthy tissue when the distal end or the margin of the leaflet is killed. ×1. B. Leaflets of purple vetch, upper and lower sides of the leaflets shown by the upper and lower leaflets, respectively. A second concentric ring having the color of the border is shown between the border and the center in some lesions. ×1. C. Leaflets of smooth vetch, the upper and lower sides of the leaflets illustrated in the upper and lower rows of leaflets, respectively. Two healthy leaflets at the left. The light leaflets had turned yellow. Dark conidial masses are in the centers of some lesions. ×1\frac{1}{4}.

a narrow border that may be Brussel's brown, raw umber, bay, or warm sepia, depending on the variety of the host attacked and other factors. Even when large areas of the leaflet are involved and the outline of the spot is lost the discolored border is present, and sometimes veins are discolored when the entire leaflet is involved. Severely affected leaflets eventually turn yellow and fall off. The colored border is not evident in the very young lesions but is soon formed and gradually develops into a well-defined narrow band adjacent to the healthy tissue. Rarely it may be more diffuse and cover a large proportion of the lesion. Sometimes there is a secondary, usually lighter, concentric ring similar to the border but nearer the center of the spot (Fig. 1, B). Under moist conditions the central area contains what appears to the unaided eye to be a large globular mass of conidia (Fig. 1, C). Examination with the hand lens or low power of the microscope shows that this mass, which usually is from \(\frac{1}{4} \) to 1 mm. in diameter, consists of many small acervuli, that are 25 to 100 µ in diameter, more commonly 30 to 50 u. Acervuli may be scattered irregularly over the lighter parts of the lesion or may be so close together that the masses of conidia coalesce. They are most common on the lower leaf surfaces, especially those of the lower leaves which may be almost completely covered by acervuli during a prolonged wet period. Conidial masses at first are nearly white or gray but soon darken to an ivory vellow, and later to a warm buff or ochraceous buff. In older spots this central area may appear nearly black because of numerous setae and black stromatic cells. Leaves killed by the fungus may have many black stromatic masses on one or both sides of the leaflets.

On young stems and petioles the lesions, at first slightly sunken, are linear, light-green areas, from 1 to a few mm. long by less than 1 mm. wide. The lesions darken with age, enlarge somewhat, seldom become more than $\frac{1}{2}$ cm. long, and often coalesce with adjacent spots until large areas of the stem may be involved (Fig. 2, B and C). The centers of the stem lesions are light and the margins are much the same color as the borders of the leaf spots. When large areas of the stem are involved the color usually approaches a warm sepia. Acervuli are borne in the centers of the younger lesions. Young stems, petioles, and tendrils may be killed. Old stems are discolored and defoliated, but whether or not they are actually killed has not been definitely established. Young stems are seldom killed by a single lesion, but if two or more lesions occur near one another, as on opposite sides of the stem, a sufficiently large proportion of the tissue may be involved to girdle the stem. Small stromatic bodies may be present on the stems as well as on the leaflets. Setae may be present in varying number or they may be wanting.

The fungus has not yet been obtained from the pods, but it seems probable that pods are attacked.

PATHOGENICITY OF THE FUNGUS ISOLATED FROM LESIONS

Isolations from the spots on the leaves and from stems have shown the constant association of an anthracnose fungus with the disease. The fungus

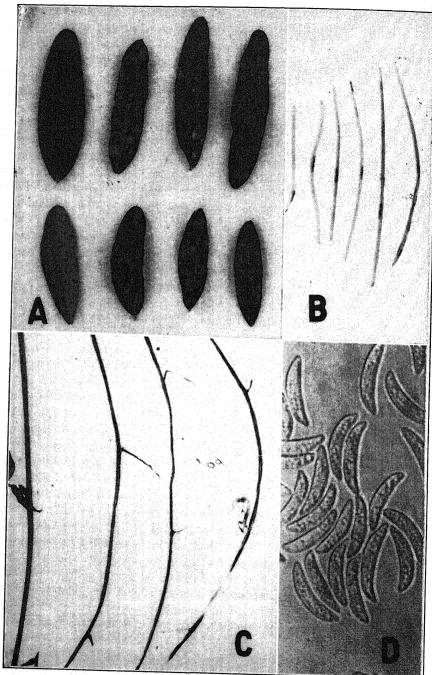


Fig. 2. A. Upper and lower sides of vetch leaflets showing early stage of infection under extremely favorable conditions. The light green lesions, with little or no colored border yet visible, often are confluent and may involve a large part of, or the entire, leaflet. ×2. B. Stems of smooth vetch seedlings with early stage of infection resulting from inoculation. ×2. C. Stems of older plants. Stem at the left is healthy, others have dark lesions resulting from inoculation. ×1\frac{3}{5}. D. Conidia of Collectrichum villosum. ×1000.

has been grown in pure culture, single spore lines obtained, and inoculations

made. The fungus obtained in culture was pathogenic on several species of vetch.

1945]

On October 4, 1943, young smooth vetch plants growing in steamed soil in pots in the greenhouse were atomized with a sterile tap water suspension of conidia from a pure culture. Control plants were atomized with sterile tap water. The plants were kept in an inoculation chamber at high humidity for 48 hours and then returned to the greenhouse bench. Six days later water-soaked spots were on some of the leaflets and soon became light green. Two days later the characteristic brown borders appeared about the spots, and typical conidial masses developed later. Some of the stems were heavily infected, while others had only a few lesions (Fig. 2, B and C). In one pot there were 61 seedlings, 24 of which had stem lesions. One month after the inoculations 4 seedlings had died from stem girdle and several others were nearly dead; whereas some showed little damage. When petioles were infected the leaflets usually died. The larger stems were seldom girdled by a single lesion, but two or more lesions on opposite sides frequently severed these seedling stems. Often there were many lesions on a stem. Isolated lesions were linear and not over 5 mm. long by about 1 mm. wide. When several lesions had coalesced, areas 10 cm. or more were sometimes involved. The lesions varied considerably in color, the borders being chocolate to almost black, and the centers light ochraceous salmon to nearly black. One to many black, raised fruit bodies, with diameters of 1-0.1 mm. or less, were scattered over the surface of the lesions.

Another inoculation experiment was started on October 18, 1943. The plants were over a month old, and were approximately the size of plants in the field when infection first becomes apparent in the spring. A heavy conidial suspension was made from two 10-day-old cultures of the fungus growing on corn-meal agar in Petri plates. Three potted plants were inoculated and one potted plant was maintained as a control. After being atomized with the conidial suspension, the plants were held for 40 hr. in a moist chamber, in which the initial temperature was 35° C. but lower at night. After 6 days the infection was evident as pale green, more or less circular, spots on the leaflets. Many leaflets were infected and a considerable number had fallen 3 days later, or 9 days after the inoculations. Many of the lesions had developed the characteristic brown borders at the junction of the diseased and healthy tissue. Some lesions had conidial masses in the center and these contained mature conidia. In many instances the healthy leaf tissue beyond the spot had turned yellow and the leaflets had fallen. Thus, considerable defoliation can occur and a new crop of mature conidia be produced within 8 to 10 days after inoculation. On the ninth day the stem lesions were linear, from one to a few mm. long, and less than 1 mm. wide. The lesions had one or more pustules bearing mature conidia. The fungus was readily recovered from the diseased tissue, grown in pure culture, and used in further inoculation tests.

POSSIBLE SOURCES OF INOCULUM

The earliest stage of the disease observed under natural conditions is the leaf-spot stage seen in early spring. Its presence in destructive amounts varies from year to year depending largely on the amount of rainfall and the amount of inoculum present. The plants are usually fairly well developed before much damage is done, but may be severely defoliated about blossoming time. The source of the early infections has not been definitely determined; but two experiments were conducted to determine if the fungus could be transmitted by seed or soil under experimental conditions.

Pots of soil were steamed and then each was planted with 25 seeds of smooth vetch. Two pots were planted with seed that were first immersed in a conidial suspension. A heavy suspension of conidia was applied to the soil in 2 other pots after the seed had been planted. Three pots were held as controls. The seeds were planted February 3, and the seedlings examined March 3, 1944. When removed from the soil, washed, and examined, 47 plants were in the 2 pots in which the seed were inoculated and all had brown to black discolorations of the stem just below the soil surface (Fig. 3, A). Distinct lesions were present, some of which were very minute, whereas others had coalesced and formed darkened areas of considerable size. No girdling had resulted. The 2 pots in which the soil had been infested had 22 plants and all had darkened stems as did those for which the seed had been inoculated. Only 2 of the 69 control plants had a somewhat similar blackening. Isolations were made from several of the blackened areas. Since lactic acid could not be used to inhibit bacterial growth, the fungus was rather difficult to isolate; and it grew so slowly that when secondary fungi or bacteria were present they invariably outgrew it. Since the infected parts were underground tissue, secondary organisms, largely Penicillia, Fusaria, and bacteria, were present in many of the platings. However, after the cultures were several days old, each plate was examined microscopically and both setae and conidia of the anthracnose fungus were found. Since inoculated seed had been planted in steamed soil and clean seed had been planted in soil infested with conidia of the fungus, and since the control plants had no infection, there seems to be little doubt that the anthracnose fungus, when present on the seed or in the soil, can attack the stems of the young seedlings. Conidia formed on these initial infections can supply inoculum for the continued spread of the fungus to other parts of

Many plantings of vetches have been observed and it seems likely that the fungus may live over in the soil or on plant débris, since the disease may be absent or present in very small amount when vetches are grown on land that has not previously grown this crop, yet it may be very severe when vetches are grown on land that has grown vetch for several consecutive years. It also is possible that the seeds become contaminated with conidia when diseased plants are threshed or that pieces of stem, leaf tissue, or thickwalled stromatic cells present on leaf blades, petioles, or tendrils might

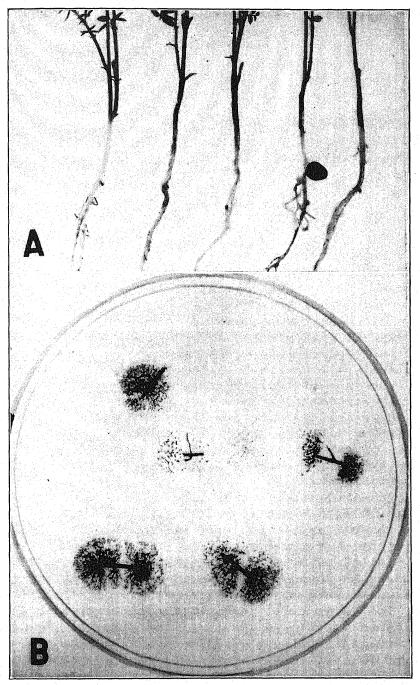


Fig. 3. A. Stems of young smooth vetch plants grown in pots in the greenhouse. The plant at the left is healthy, the next 2 plants to the right have dark lesions, on the stems just below the soil surface line, resulting from infection in artificially infested soil. The 2 plants at the extreme right have similar lesions resulting from seed inoculation. $\times 1_{10}^{+}$. B. The fungus growing from surface-disinfected, diseased stems on corn-meal agar. $\times 1_{12}^{+}$.

be mixed with the seed and carried to the field. Volunteer plants of smooth and hairy vetch often are widespread in vetch-growing sections and it is probable that such plants can harbor the fungus and be a source of inoculum. No perfect stage of the fungus is known. Since one crop of vetch matures in late May or June and the next is planted in September or October, no prolonged period for the survival of the fungus outside of the host is necessary.

CULTURAL CHARACTERS AND MORPHOLOGY OF THE FUNGUS

Some difficulty was experienced in isolating the causal fungus from the tissue and even from conidial masses. The fungus would not grow on a strongly acidified medium, as, for example, when 1 or 2 drops of 50 per cent lactic acid were added to 15 ml. of medium to inhibit bacterial growth. Several other anthracnose fungi, from legumes, grew readily on such an acidified medium. Some of the early studies were attempted during the summer months and no growth was obtained because of too high temperature. Once the acidity and temperature factors were corrected the fungus could readily be isolated from stem (Fig. 3, B) or leaf tissue and grown on different culture media. The fungus grew well and sporulated on corn-meal and oatmeal Two per cent potato-dextrose agar and fresh, green-string-bean pods proved to be excellent media for production of conidia. Canned beans were much less favorable, and nutrient agar was unsuitable. At best the fungus grew slowly. It produced a white mycelium which later turned dark brown, and became thick-walled and much septate (Fig. 4, M). On old corn-meal agar slants there were many small irregular masses of dark, thick-walled cells often buried deeply in the agar. These sometimes were so abundant as to give the medium a dark color. On the surface of the agar many gray to pale-salmon conidial masses were formed, with or without setae scattered among them. One or more setae sometimes were present in or near the center of each acervulus.

Germination of conidia was studied in some detail on corn-meal-agar plates. Conidia from a 10-day-old culture on corn-meal agar were suspended in sterile tap water, then poured over the surface of the agar. The earliest sign of germination was noted in 4 hours at room temperature (25–30° C.). About 50 per cent of the conidia had germinated in 24 hours and germ tubes were from 1 to 5 times as long as the conidia. A single germ tube was formed at or near one end, more rarely at the side, of the conidium (Fig. 4). The germinating conidium usually became septate very early. In some cases a brown appressorium was formed on the germ tube a short distance from the conidium, and this later sent out a tube that penetrated the agar (Fig. 1, D). On corn-meal agar, however, the germ tube more commonly continued to elongate, and after 24 hours conidiophores began to develop. A small swelling first appeared on one side of the germ tube a short distance beyond the conidium (Fig. 4, E), continued to enlarge, and became a conidiophore varying in length from 1 to 5 times, but more often

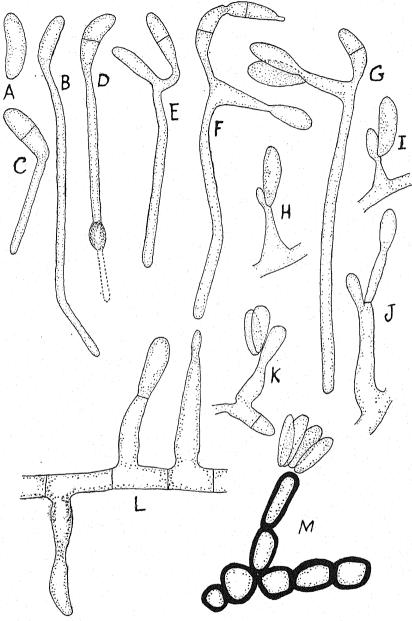


Fig. 4. Different stages of conidial germination on corn-meal agar. A. Not germinating. B, C, and D. Early stages of germination. D. An appressorium a short distance from the conidium. E. Conidiophore forming on the germ tube near the conidium. F. Slightly more advanced stage than in E. Conidium forming on the young conidiophore and a secondary conidium, also starting to germinate, formed directly from the other cell of the conidium. G. Slightly later stage than in F. The first conidium formed has been displaced by a second one. H, I, and J. The second conidium formed by a conidiophore pushes aside the first conidium which eventually falls off. K. Short conidiophore formed directly from one cell of the conidium. L. The multicellular germ tube, with each cell sending out a conidiophore which bears numerous conidia. M. Thick and dark walls of the old mycelium that forms black stromatic layers. Even the walls of the conidiophore are thick and dark. All drawings approximately ×1000.

2 to 3 times, as long as the conidium. The conidiophore usually was slightly wider at the base than the germ tube and often was slightly constricted at the point of attachment. It tapered to approximately the size of the conidium at the distal end. A single conidium formed at the apex of the conidiophore and as this matured a second conidium formed (Fig. 4, H and I). The first conidium was finally shoved aside and dropped off as its place was taken by the second conidium (Fig. 4, G and K). This process continued so that eventually a considerable number of conidia accumulated on the agar at the end of the conidiophore (Fig. 4, G, K and M), very much as described and illustrated for cotton anthracnose by Atkinson.3 The germ tube continued to elongate and sometimes reached a length of 500 to 1,000 u in 72 hours, and it finally branched to form a small mycelial mat in 90 hours. A conidiophore was produced from most of the cells thus formed, and each conidiophore formed numerous conidia. In this manner large quantities of conidia were produced as the result of the growth of a single conidium. As many as 50 conidiophores were counted on a single germ tube 72 hours old. After the first germ tube was well developed the other cell of the now septate conidium germinated. A short germ tube usually was formed and served as a conidiophore. Sometimes a typical conidiophore or a secondary conidium with no perceptible germ tube formed directly from either cell of the germinating conidium (Fig. 4, F and K).

Young mycelium was hyaline and 2–4 μ in diameter. Later the mycelium became closely septate, the cells were more rounded, sometimes 10 μ or more in diameter, the walls became thick and brown, and thus a stromatic mass of cells was formed (Fig. 4, M). Even the conidiophores became dark and thick-walled. Setae might be interspersed among the thick-walled cells. One of the thick-walled cells sometimes served as the basal cell of the seta.

Conidial germination was studied first in tap water. Short germ tubes were usually produced by only a small proportion of the conidia, and then growth ceased. Apparently some condition suitable for continued growth was missing. Since conidia placed in the same tap water and then atomized on the host gave good infection, it was thought that juice or particles of the host might stimulate growth of the conidia in tap water. This was the case, since when pieces of tissue or juice of several species of vetch were mixed with a suspension of conidia in sterile tap water considerable increase in germination and in vigor of the germ tubes was apparent. In some cases germination in the water controls was about 10 per cent; when vetch juice or tissue was added germination varied from 75 to 95 per cent.

EFFECT OF TEMPERATURE ON THE FUNGUS

A knowledge of the temperature relations of *Colletotrichum villosum* is essential if it is to be cultured successfully. The fungus failed to grow in the laboratory during the summer because of too high temperatures, and Atkinson, G. F. Anthracnose of cotton. Jour. Mycol. 6: 173-178. 1890-91.

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cultures were lost unless held in a refrigerator. The following summary of the temperature studies will serve as an aid in culturing the organism.

The fungus was grown on 2 per cent potato-dextrose agar in Petri dishes held at different temperatures and the results were recorded at the end of 2 weeks. A few conidia germinated at 3.5° C., and at 13.3° C. germination was good but no macroscopically measurable colony had been formed. The upper limit for growth was near 32 and the optimum lay between 24 and 26° C. Growth was fair at 21.5 and 29° C.

DESCRIPTION OF THE CAUSAL FUNGUS

This anthracnose disease has certain characters in common with the disease caused by Kabatiella nigricans. The symptoms on the stems, at least to one not familiar with both diseases, can readily be confused. The conidia of the anthracnose fungus and those of K. nigricans are very similar both in size and shape. The leaf symptoms, however, are different. Lesions of K. nigricans are illustrated and described by Wolf⁴ as "circular with a tendency toward being most numerous along the principal veins or . . . as elongated, dark streaks." A study of conidia production and germination shows clearly that the anthracnose fungus differs from K. nigricans in that in the former the conidia are borne singly on conidiophores, about the width of the conidium at the point of attachment instead of on the large conidiophore characteristic of Kabatiella. Likewise there is no budding of the conidia on germination as there is with K. nigricans.

The literature dealing with closely related diseases of vetch is reviewed by Wolf.* He mentions Gloeosporium davisii Ell. and Ev. and G. everhartii Sacc. et Syd. These possess smaller and differently shaped conidia than does the fungus studied by the writer. The same is true of G. viciae Fautr. et Roum. G. tricolor Lind has much longer conidia than does the fungus of this paper. The literature on the anthracnoses of other legumes has been surveyed and so far as could be ascertained all the causal fungi are different from the vetch anthracnose fungus considered here.

Perhaps the fungus most similar to the one studied by the writer is Colletotrichum viciae Dearn. and Overh. Dr. Overholts kindly sent the writer a specimen of his material and a study of this seems to leave little doubt that the two fungi are different. For example, C. viciae does not form definite circular lesions with colored borders on the leaflets but a part or all of the leaflet is affected, and accervuli are scattered promiscuously over the affected tissue just as described by Dearness. The accervuli are more numerous on the upper side of the leaflets affected by C. viciae, which is just the reverse from that in the Georgia material. The conidia of the writer's species average about the same as the maximum width of C. viciae. The setae of C. viciae are shorter and narrower and have only 1 septum instead of 1 to 4. It seems to the writer that these differences in symptoms

⁴ Wolf, F. A. A little-known vetch disease. Jour. Elisha Mitchell Sci. Soc. 36: 72–85. 1920.

⁵ Dearness, J. New and noteworthy fungi-V. Mycologia 20: 235-246. 1928.

produced in the host together with the location of the acervuli most commonly on the upper side of the leaflets, the differences in conidial width, and in size and septation of the setae, are sufficient to justify the conclusion that *C. viciae* is a different species from the one studied by the writer.

Since the fungus attacking vetch in Georgia appears to be different from any described in the literature, it is considered a new species and the name *Colletotrichum villosum* is proposed.

TECHNICAL DESCRIPTION

Colletotrichum villosum n. sp.

Acervuli in foliis amphigeni, saepius hypophylli, 25–100 μ in diam.; massae conidicae griseolae usque ochraceo-bubalinae; setae carentes vel abundantes, hyalinae usque rubro-brunneae, 45–150 \times 6.3–9.5 μ ad basin bulbosam, attenuatae, 1–4-septatae; conidia hyalina, subcurvata, unicellularia, 15–24 \times 3.5–4.5 μ .6

Colletotrichum villosum sp. Lesions typically more or less circular to linear, light brown to gray with Brussel's brown to warm sepia border, becoming dark brown to almost black on stems, amphigenous, 1–2 mm. in diameter. Acervuli amphigenous, more commonly hypophyllous on leaflets, 25 to 100 μ in diameter, more frequently 30 to 50 μ , or larger and irregular when confluent. Conidial masses gray to warm buff to ochraceous buff. Setae wanting or abundant, variable from hyaline to purple-gray to red-brown, approaching Brussel's brown of Ridgway, becoming lighter at the tips and often at the base, average 85 μ (range 45 to 150 μ) long by 7.5 μ (range 6.3 to 9.5 μ) wide across the bulbous base, tapering to a rounded point, 1 to 4 septate. Conidia hyaline, slightly curved, bluntly tapered, unicellular, average 18.9 by 4 μ (range 15–24 \times 3.5–4.5 μ) 18 to 20 μ most common length.

Habitat: Parasitic on leaves and stems of Vicia villosa Roth., V. sativa L., V. atropurpurea Desf., V. grandiflora Scop., V. monanthos (L.) Desf., V. dasycarpa Ten., V. angustifolia L., and V. pannonica Crantz in Georgia and Florida, U. S. A.

Material has been deposited in the herbaria of Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Md., Department of Plant Pathology, Cornell University, Ithaca, N. Y., and Department of Botany, Georgia Agricultural Experiment Station, Experiment, Ga. No. 71420 (Myc. Coll. Bur. Pl. Ind.) collected by J. L. Weimer, Experiment, Ga., on *Vicia villosa* Roth, May 6, 1943, is designated as type.

CONTROL

Although little experimental work has been done on the control of this disease, several methods may be possible. The use of clean, disease-free seed, preferably obtained from areas where the disease does not exist, or where environmental conditions are not suitable for its rapid development, should reduce the possibility of distributing the fungus, or at least delay its accumulation in the soil of any field not already heavily infested. Evidence available at present suggests that the disease seldom becomes destructive if the crop is not grown on the same land continuously. This means that rotation properly managed may be a fairly satisfactory control measure. The destruction of volunteer vetch in the vicinity of vetch fields will eliminate another source of inoculum.

One means of controlling such field-crop diseases is the use of resistant varieties or closely related species that might be suitable as crops. In order

⁶ Latin diagnosis supplied by Edith K. Cash, Division of Mycology and Disease Survey, Bureau Plant Industry.

to determine if any of the common vetch varieties or species were resistant, several lots were grown in pots in the greenhouse and inoculated by atomizing with a conidial suspension. The ratings of the different lots tested in two experiments were based on a 0 to 10 scale (Table 1). The lots of *Vicia sativa* were fairly resistant for the most part. They were not immune, since most of them had a large number of lesions, but these remained small

TABLE 2.—The relative resistance of some vetch varieties and species to anthracnose. Rated on a scale of 0 to 10

·Variety or species name and	Rati	ng in
F.C. or other number	Experiment 1	Experiment 2
Vicia sativa, Selection 5	2	3
7		$ar{2}$
103	5	
F.C. 16462	1	2
Vicia sativa alba, F.C. 02830	2	3
F.C. 18808	2	3
F.C. 81566		1
F.C. 18052		
F.C. 18056-1	2	
F.C. 18814		4
F.C. 18818		
F.C. 22785		
F.C. 22790		
F.C. 29933		2
F.C. 30171		-
F.C. 31361		
F.C. 31542		2
F.C. 31543		
F.C. 34947		4
F.C. 11725		
F.C. 119988		*****
F.C. 119994		4
F.C. 31546		3
F.C. 31545		i
V. atropurpurea Desf.		10
V. grandiflora Scop.		1
V. villosa Roth.		10
V. monanthos (L.) Desf., F.C. 18139		3
V mananthan atrain not numbered	±	3
V. monanthos, strain not numberedV. dasycarpa Ten., F.C. 22471	10	9
V. angustifolia L., F.C. 04315	10 2	4
		1
V. pannonica Crantz	4	7

^a The writer is indebted to Mr. Roland McKee, Division of Forage Crops and Diseases, Bureau of Plant Industry Station, Beltsville, Maryland, for all of the seed used.

^b Rating: 0 = no disease; 5 = moderate disease; 10 = severe disease.

and superficial, and in many cases were confined largely to the stems. The only lots severely injured in these experiments were those rating 8 to 10, namely *V. atropurpurea*, *V. villosa*, and *V. dasycarpa*. Just what would have happened to the other lots had they been subjected to a long rainy spell, as often happens in the field, is not known. However, in hotbeds where these lots were outdoors for 5 months without artificial inoculation but growing in field soil in which vetch had been grown for several years, only *V. villosa* and *V. atropurpurea* were severely damaged by anthracnose.

Also, the lots of *V. sativa* have never been observed severely damaged in the field, even when *V. villosa* nearby was largely defoliated. It may be concluded, therefore, that it is possible to control vetch anthracnose by growing one of the lots of *V. sativa* rating 3 or less, or any of the other species listed in table 2, except the 3 rating 8 or above. At the conclusion of these experiments most plants in these 3 high-rating lots were dead, the stems as well as the leaflets having been killed. In a few cases where the stems were killed, new secondary stems were formed near the surface of the soil. Presumably in the field these shoots would have been infected and possibly killed if environmental conditions were suitable for the spread of the fungus.

SUMMARY

An anthracnose leaf spot of vetches is caused by Colletotrichum villosum n. sp. On the leaves the fungus produces small circular spots at first light green, later becoming light brown or gray with a brown to red border. The stem lesions are linear and usually dark to black. Severe defoliation and death of young stems may occur during wet weather. The fungus grows slowly on nonacid culture media and fruits abundantly on sterilized, fresh string-bean pods, and on potato-dextrose, corn-meal, and oat-meal agars. The optimum temperature for growth lies between 24° and 26° C., some growth takes place at 3.5° C., and the maximum for survival is near 32° C. Vicia atropurpurea, V. villosum, and V. dasycarpa are the most susceptible of the species tested, whereas most strains of V. sativa, V. grandiflora, V. monanthos, V. angustifolia, and V. pannonica are fairly resistant. The use of disease-free seed, rotation, and the use of resistant varieties are suggested as control measures.

DIVISION OF FORAGE CROPS AND DISEASES,
U. S. DEPARTMENT OF AGRICULTURE,
AND
GEORGIA AGRICULTURAL EXPERIMENT STATION,
EXPERIMENT, GEORGIA.

A LINE-PATTERN VIROSIS OF SHIRO PLUM¹

R. S. WILLISON

(Accepted for publication July 14, 1945)

Early in the summer of 1938, symptoms suggestive of virus origin were observed on a number of trees of the Japanese plum variety, Shiro, in a 20-year-old block near Port Dalhousie, Ontario (3). The older leaves were marked with a creamy white vein-banding (Fig. 1, E), but the younger leaves were apparently normal. Symptoms persisted on the older leaves throughout the summer. Preliminary indexing of peach seedlings with bud material taken from the affected trees in September, 1938, indicated that the condition was both bud-perpetuated and graft-transmissible.

Further inquiry disclosed the fact that, some years before, a number of trees in the block had been grafted with shoots of another Japanese variety, locally known as First, in order to provide for pollination of the Shiro bloom. Since signs of the disorder were found on almost every tree thus grafted and on a number of others besides, it was quite obvious that the virosis had been introduced into the Shiro block by means of the First grafts and that it had been spread by natural means. Accordingly, the scion material was traced to its source in another orchard, in which many of the First trees had foliar patterns so mild that they would readily escape observation, and in which a number of Shiro trees displayed typical symptoms (3). The only other known case of natural occurrence in the Niagara district was found near Beamsville on Italian prune, in combination with prune dwarf (10).

The disease has been called Shiro line-pattern mosaic (3) or Shiro plum line-pattern mosaic (7), but hereafter will be referred to as line pattern. The disease has been transmitted from Shiro to a number of plum, cherry, and peach varieties, and the present paper is concerned mainly with the symptomatology of line pattern on these several hosts. The relationship of the causal virus to some others affecting stone fruits is also discussed to a limited extent.

DIFFERENTIAL VARIETIES

The range of differential hosts employed in these experiments comprised the following species and varieties: Prunus salicina var. Shiro, Abundance, and Early Golden; P. domestica var. Italian prune, German prune, Imperial Gage, Reine Claude, Lombard, and Grand Duke; P. cerasifera, Myrobalan seedlings; P. persica var. Elberta, Rochester, and seedlings; P. armeniaca var. Niagara; P. avium var. Black Tartarian and Napoleon; P. cerasus var. Montmorency; and P. mahaleb, seedlings.

PROCEDURE

Both the double-budding technique and direct inoculation of orchard trees and nursery stock by budding have been employed. Since not only

¹ Contribution No. 821 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

the techniques but also their respective advantages and disadvantages have been discussed in recent articles (10, 11), there is no need for further comment here, except to say that the symptom sequences of line pattern, in contrast to those of tatter leaf (11), were not influenced appreciably by the mode of inoculation. Like prune dwarf (10), line pattern is very easily transmitted by budding, in fact, there have been no failures with any host at all susceptible to the virus, even when union between diseased bud and healthy stock was doubtful.

SYMPTOMATOLOGY

On the susceptible hosts, symptom expression of line pattern varied considerably in degree from year to year, being both more intense and more extensive for the variety when the weather remained cool for considerable periods in the early part of the growing season than when warm weather prevailed at that time. Some differences in pattern detail were also noted in different years. Almost invariably, symptoms appeared only on the leaves emerging in the spring. No distinctions could be drawn between the acute and chronic phases of the disease (11), except possibly on Montmorency, and, up to the present, there have been no indications that vigor was impaired to any appreciable extent.

On Shiro Plum.—The symptoms on Shiro were of two main types: yellow vein-banding (Fig. 1, E), and well-defined, brilliant, green-yellow patterns, usually of the oak-leaf type (Fig. 1, C), formed by single or multiple, irregular lines or bands. In early summer, the yellow of the patterns faded to a creamy white, which persisted for the remainder of the season. In most years, a regular sequence of pattern types could be traced, commencing with the oak-leaf and followed in successive leaves by a mixture of oak-leaf and vein-banding (Fig. 1, D), by over-all vein-banding and by vein-banding towards the leaf tips. New leaves emerging after early June were symptomless. In some years, one or other of the symptom types was more or less suppressed, depending upon conditions at leaf emergence.

On Abundance Plum.—Both in double-budding experiments and on an inoculated orchard tree, the symptoms on Abundance leaves somewhat resembled those on Shiro but were always much fainter and less numerous. Vein-banding was occasionally seen, but the predominant characteristic was the oak-leaf pattern, frequently in the typical form, but sometimes represented only by a narrow line either close to the margin near the leaf tip or on the serrations. On Abundance, the patterns were generally yellowish-green and more or less transient.

On Early Golden Plum.—No symptoms were observed in four years on Early Golden growing on a double-budded Myrobalan seedling, even though the Shiro shoot on the same stock showed striking symptoms and the Myrobalan shoot was moderately affected.

On Italian Prune.—When inoculated either by double-budding or directly on nursery stock, Italian prune either had inconspicuous green oak-leaf patterns or was symptomless, depending on whether or not the season was favor-

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able for symptom expression. Line pattern was transmitted through artificially infected Italian prune to healthy Shiro without any apparent loss in virulence.

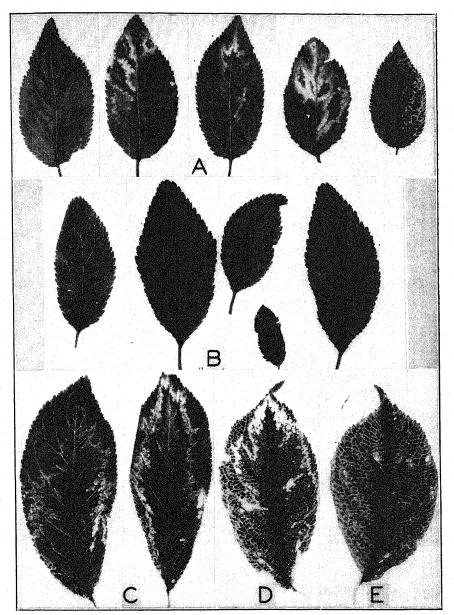


Fig. 1. Symptoms of line pattern on plums. A. Various markings on Myrobalan seedlings. B. Chlorotic and necrotic lines on Lombard. C, D, and E. Oak-leaf patterns, oak-leaf and vein-banding, and vein-banding, respectively, on Shiro.

On German Prune.—Inoculation by double-budding induced variously placed, fine, yellow lines or very irregular, spiky, oak-leaf patterns on the

early leaves of German prune. Symptoms on this variety were less vivid than those on Shiro, and also persisted throughout the season.

On Imperial Gage Plum.—One orchard tree, inoculated in September, 1938, has shown no symptoms since that time. The Shiro buds used for the purpose produced shoots having typical but comparatively mild symptoms.

On Reine Claude Plum.—Symptoms on Reine Claude, double-budded, were always mild, though slightly more conspicuous and numerous than those on Italian prune. Patterns were usually of the oak-leaf type, and were sometimes reduced to a narrow line near the tip of the leaf (Fig. 2, D). The markings were sometimes very slightly yellower than the normal leaf, but were usually best visible by transmitted light.

On Lombard Plum.—Early leaves near the point of inoculation, on nursery stock or trees, were occasionally and comparatively faintly marked with oak-leaf patterns, fairly large irregular rings, or vein-banding. More frequently and characteristically, a chlorotic line, which later became necrotic, outlined a triangular sector. The base of this sector lay at the leaf margin and the apex at or near the central vein (Fig. 1, B). Leaves marked in this fashion were usually distorted. In general, only a few leaves showed symptoms of any kind in any one year, and the disease seemed to spread slowly through individual trees.

On Grand Duke Plum.—No inoculations with line pattern alone were made on Grand Duke, but, in a series double-budded with the line pattern-prune dwarf mixture (10), leaves were conspicuously marked with fine lines, yellow oak-leaf, or large, irregular, yellow rings, which sometimes became partially necrotic. Since these patterns did not appear when Grand Duke was infected with prune dwarf alone, they are considered to have been caused by the line-pattern component of the mixture. The necrosis observed in Grand Duke leaves may have been due to the presence of the prunedwarf virus, as in the Montmorency series described later. In the mixture, each virus appeared to aggravate the effects of the other on several varieties of the differential hosts used.

On Myrobalan Seedlings.—Individual seedlings of Myrobalan differed widely in their reaction to the line-pattern virus. At one extreme were the resistant or tolerant plants on which very few patterns were evident even in seasons most favorable for symptom expression. On these, symptoms, if they appeared at all, were usually a whitening of the tips of the marginal serrations and occasionally yellow-green oak-leaf patterns. At the other extreme were the susceptible seedlings on which the markings were usually indistinguishable from those on Shiro, in color, form, and distribution. Most Myrobalan plants, however, were only moderately susceptible. Some of the varied patterns to be seen on Myrobalan are illustrated in figure 1, A.

On Peaches.—Since the line-pattern symptoms expressed by the different peach varieties and seedlings varied little in quantity and less in kind, one general description will suffice. The most common symptom was a fine, irregular, wavering line on each side of the main vein of a leaf (Fig. 2, B),

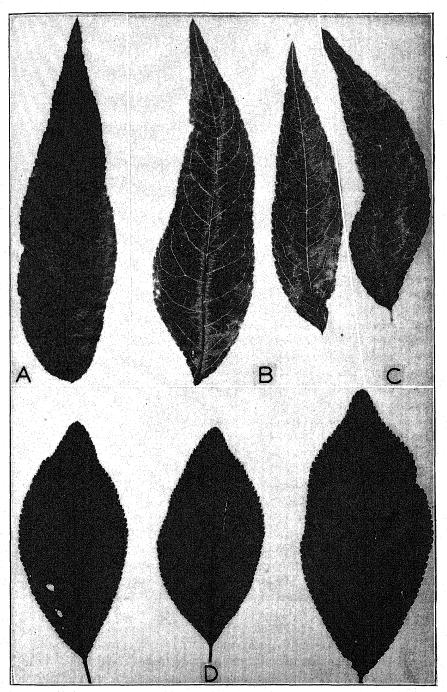


Fig. 2. Symptoms of line pattern on peach and plum. A, B, and C. Fine network, symmetrical lines, and broken lines, respectively, on peach. D. Faint oak-leaf and line patterns on Reine Claude, photographed by transmitted light.

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placed anywhere between the mid-rib and the margin. The lines on a single leaf usually formed a symmetrical pattern but sometimes one was either broken or turned back on itself to form figures of various shapes (Fig. 2, C). Occasionally leaves were marked with a network of fine lines forming irregular patterns (Fig. 2, A), with fine, confluent rings, or with vein-banding. On the peach, patterns were mostly pale green, and tended to disappear later in the summer. The majority of leaves, especially those emerging later than early June, were symptomless. The number of leaves affected and sometimes the predominant symptom type varied from year to year according to growing conditions in the spring. In exceptionally favorable seasons, some yellow vein-banding and oak-leaf may appear.

On Apricot.—Up to the present time, no definite symptoms of line pattern have appeared on the apricot variety, Niagara, used in double-budding experiments, in spite of the fact that the disease was transmitted to the peach seedlings used for stock.

On Sweet Cherries .- The two varieties, Black Tartarian and Napoleon, responded in much the same way to inoculation with the line-pattern virus. Some of the symptoms resembled those induced by prune dwarf (10) or tatter leaf (11), for example, tiny, fine rings (Fig. 3, I), or larger, coarser rings and faint oak-leaf (Fig. 3, H). These patterns were pale green and tended to disappear in the course of the summer. Two symptoms, however, were peculiar to line pattern. The first was an inconspicuous line almost like a watermark, separating two areas of the leaf with slight differences in color. The second, and more common, was a series of yellow lines, varying from a hair's breadth to about 1 mm. These patterns were usually restricted to a small fraction, seldom more than a quarter, of the leaf surface. The finer lines frequently ran parallel to the secondary and some of the finer veins (Fig. 3, E), whereas the coarser ones delineated irregular patterns (Fig. 3, D). As on Shiro plum, the yellow markings persisted, turning creamy white later in the summer. The symptoms appeared in the vicinity of the inserted buds the first season but were more widely scattered in subsequent years. Most leaves were symptomless.

On Montmorency Cherry.—Symptoms on Montmorency nursery stock, in the first season after inoculation, were pale green markings in the form of broad bands, spots of various sizes, and coarse rings up to about 6 mm. in diameter (Fig. 3, A). The borders of these patterns were ill-defined and faded out into normal tissue. On a few leaves, a fairly coarse, irregular, yellow ring or partial ring appeared near the tip. A few, scattered, necrotic spots were observed at the bases of a number of affected leaves.

In June of the second year, leaves were marked with narrower and more sharply defined lines, variously placed with respect to the mid-rib (Fig. 3, B). These lines, though occasionally faintly tinged with yellow, were usually more like watermarks, more readily discernible by transmitted than by reflected light. Identically similar lines appeared at the same time on leaves of Montmorency inoculated with the prune dwarf-line pattern mix-

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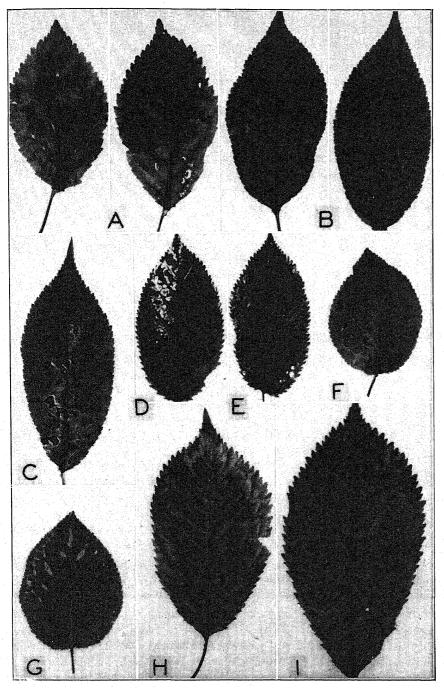


Fig. 3. Symptoms of line pattern on cherries. A and B. First and second season patterns, respectively, on Montmorency. C. Translucent lines and necrotic spotting on Montmorency affected with the line pattern-prune dwarf mixture. D. Coarse, yellow pattern, on Napoleon. E. Fine, yellow lines, on Black Tartarian. F and G. Partly modified oak-leaf, and short, yellow vein-bands, with necrosis of veins, respectively, on Mahaleb. H. Pale green lines, on Napoleon. I. Pale green rings, on Black Tartarian.

ture (10), but soon either went over into a dark, red-brown necrosis, or became associated with irregular necrotic spotting (Fig. 3, C). The necrotic phase did not appear with line pattern alone, and was evidently attributable to the combined effects of the two viruses.

On Mahaleb Seedlings.—In early spring, some Mahaleb leaves were marked either with oak-leaf patterns (Fig. 3, F) or with coarse rings and lines, sometimes darker and sometimes paler than normal. Of greater diagnostic value were the short, narrow, yellow bands which appeared along some of the veins of a number of leaves (Fig. 3, G) scattered over the plant. In these areas, the vein and, frequently, part of the yellow band eventually became necrotic, and either split or dropped out. In the early fall of the first season, a few of the newer leaves developed either coarse, pale, oak-leaf patterns or huge rings.

DISCUSSION

A comparison of the descriptions of the disease, as it appears on the different hosts, reveals a fundamental uniformity underlying the array of patterns, in so far as they can be referred to a linear type of variegation. Within the limits of that general type, however, considerable diversity of form was displayed, even on individual plants; oak-leaf, vein-banding, single and multiple lines and bands, and rings of various sizes. Although there was a tendency for one or other of these forms to predominate in a given species or variety of host, varietal reaction was largely a matter of intensity of expression. In this respect, there was a whole gamut of variation ranging from the barely perceptible patterns on First, Italian prune, and Reine Claude, through the green markings on peach and cherry leaves, to the brilliant yellow and cream figurations on Shiro and other hosts, and even to slight necroses on Mahaleb, Montmorency, and Lombard leaves. This suggests different levels of resistance and susceptibility on the part of the several host varieties. In this regard, the plum varieties of the three species, Prunus domestica, P. salicina, and P. cerasifera, were more variable than the peach and cherry varieties. It may be of interest here to note that varietal differences in susceptibility are not the same for line pattern as for either prune dwarf (10) or tatter leaf (11).

The intensity of symptoms varied not only with variety but with season. Observations over a six-year period lead to the conclusion that symptoms are suppressed in those leaves which develop when summer temperatures prevail. Though no accurate determinations of the temperature relations involved have been made, circumstantial evidence would indicate daily mean temperatures of 55° to 60° F. as critical for symptom expression. The possibility that other external and internal factors may also affect the virus-host relationship from year to year should not be overlooked.

Apart from growers' statements that crop yields of Shiro were apparently unaffected by line pattern, few data on that phase of the disease are yet available. However, fruits on infected Rochester and Elberta trees were normal in shape and quantity in 1944. Line pattern has also had little effect

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upon vigor of growth to date, but whether it induces a gradual decline in vigor and crop production remains to be seen.

Present information would indicate that line pattern is not widely distributed in the Niagara district, since it has been found only in three orchards, and, although easily transmitted by grafting, has not yet turned up in masked form on plums indexed at the laboratory. Some evidence of natural dissemination in two of the affected orchards has been mentioned. Consequently, the existence of an insect vector may be postulated, but the infrequency of natural occurrence in the district and the absence of natural spread in the laboratory plantation during the last six years would suggest not only that the vector is comparatively rare but also that there is no transmission by sap transfer during pruning. The amply demonstrated possibility of inadvertent transmission of line pattern, as well as other viroses, through the use of scions in which the disease is more or less masked, emphasizes the necessity of great care in the selection of propagating material.

That some of the symptoms of line pattern on cherries and Mahaleb have much in common with some symptoms of tatter leaf and prune dwarf, indicates that there is some overlapping of the symptom expression of a number of distinct viroses on certain hosts. On the other hand, a number of characteristic symptoms, seasonal peculiarities, and differential host reactions serve not only to separate the line-pattern virus distinctively from other viruses under investigation here, but also to link it with viruses causing similar characteristic reactions elsewhere.

Through the courtesy of Donald Cation in supplying the necessary scions, the line-pattern virus that he worked with in Michigan (4) has been transferred to most of the differential hosts employed in Ontario. The symptoms of the Michigan virus on the respective differential hosts so resembled those induced by the native virus from Shiro that there is no reasonable doubt that the two viruses are very closely related, if not identical.

The virosis transferred by Valleau (9) from Abundance 422, Red June, and October Purple plums to peach (Valleau's figures 19, 22, and 23) as well as the one he found on Abundance in a nursery in 1931 (Valleau's figure 24) also appear very similar to those under observation in Ontario. Valleau's report that no patterns were seen on Shiro (9), leaves the identity of his virus still in question, and suggests either a difference in strain or the suppression of symptoms on Shiro under Kentucky conditions. The destruction of Japanese plums in Kentucky (7, p. 66), attributed by Valleau to ring spot or line pattern, is at variance with other reports on line pattern—like viroses, and may well have been caused by a mixture of viruses. Cation's experiments with plums from Kentucky (4) indicate that more than one virus was involved, and an increase in the virulence of line pattern when mixed with another virus has been noted.

Hildebrand (6) observed line pattern symptoms on Red June and Abundance in New York state, but his data are not sufficient to permit further comparison.

The symptoms on Santa Rosa plum of the "Vacaville plum mosaic." which Thomas and Rawlins (8) report as being similar to the Kentucky ring spot, appear to be of the line pattern type, but their description of the symptoms on peach as "consisting in mild mottling without any definite pattern" is not in agreement with the findings in Michigan (4) and Ontario. This again may be the result of differences either in virus strain or in environmental conditions. On the other hand, Thomas and Rawlins also refer to a second virosis on Santa Rosa and Duarte plums, symptoms of which on Myrobalan, according to description, seem to correspond well to those noted in Ontario on the same host. There is also a remarkable resemblance between the symptoms of banded chlorosis of Japanese cherries in Oregon, as described by Zeller and Milbrath (12), and those of line pattern on Shiro and Myrobalan in Ontario. It is, perhaps, more than coincidence that line pattern occurs in nature on Japanese varieties of plums in Kentucky, Michigan, New York, Ohio, and Ontario, that similar diseases were found on Japanese plums in California, and that banded chlorosis is common on Japanese cherries in Oregon.

References to line pattern-like virus diseases on stone fruits are also to be found in the European literature. G. and M. Arnaud (1) do not give any illustrations, but their general description of the symptoms of a mosaic on Myrobalan, apricot, and peach is equally applicable to the Ontario line-pattern virosis. On the other hand, the French virus differs from the Canadian one in that it can affect apricot but not Mahaleb. This would suggest strain differences in related viruses, though the different results with apricot could just as well be explained on the basis of varietal differences in the host.

Christoff's illustrations of narrow-striped variegation and ring-spot variegation (5) on Myrobalan and plum in Bulgaria show great similarity to the line-pattern symptoms on Myrobalan and Shiro. Furthermore, the symptoms of the Bulgarian disease on peach are described as light green to yellow thin lines and little rings. Atanasoff (2) also reported and illustrated a similar striped variegation on peach in Bulgaria. His virus induced yellow to white patterns on peach and distortion of fruit and in these respects differed from the Canadian one.

The viroses of the line pattern type, reported on stone fruits at widely separated points in North America and Europe, are then sufficiently alike in symptom expression to form a distinct group of diseases. Furthermore, some of them are reported as inducing symptoms only on leaves formed in the spring (1, 4, 5), and most do not greatly affect the vigor of the host (1, 4, 5, 8, 12). Since there is evidence that the causal viruses are not identical, it is suggested that a number of strains of the line-pattern virus are implicated. There also remains the possibility that some of the discrepancies may arise from contamination with other viruses, or from differences in environment.

SUMMARY

A striking yellow to white line-pattern virosis, found originally on Shiro

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plum, has been transferred by budding to numerous species and varieties of the genus Prunus.

On all susceptible hosts, symptoms consisted of linear markings in diversified patterns, such as rings, lines, bands, oak-leaf, and yein-banding. These patterns occurred only on leaves produced in the spring, but persisted usually throughout the summer.

Symptoms varied in intensity and extent on the same host from year to year, being more plentiful when cool weather prevailed in the early part of the growing season.

Intensity of symptoms also varied greatly in plum varieties and Myrobalan seedlings. Patterns were virtually absent in Early Golden and Imperial Gage, barely perceptible and translucent in Italian prune, Reine Claude, and First, and brilliant yellow to white in Shiro and some Myrobalan seedlings. On Lombard, many of the patterns became necrotic even though comparatively few leaves per tree were affected.

On peaches, which did not show any wide differences in varietal susceptibility, symptoms usually took the form of pale green, irregular lines, rings, and vein-banding.

Faint, green rings and lines and yellowish to white patterns were found on the sweet cherry varieties, Black Tartarian and Napoleon. On the sour cherry variety, Montmorency, diffuse pale streaks, spots, and rings, and slight necrotic spotting appeared in the first, and narrow, translucent lines in the second season after inoculation. Mahaleb seedlings showed green rings and lines, as well as short, yellow, later necrotic, streaks along some of the main veins.

The writer wishes to express his sincere thanks and appreciation to Dr. G. H. Berkeley for much helpful advice and constructive criticism during the course of the investigation.

DOMINION LABORATORY OF PLANT PATHOLOGY, ST. CATHARINES, ONTARIO, CANADA.

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TWO ALTERNARIA DISEASES OF CRUCIFEROUS PLANTS1

JEFFERSON F. RANGEL²

(Accepted for publication July 18, 1945)

An ever-increasing number of Alternaria species are being described on cruciferous plants. The two which have received the most attention are Alternaria brassicae (Berk.) Sacc. causing black leaf spot and A. herculea (Ell. & Mart.) Elliott, producing the grey leaf-spot disease. These two generally are present on mature plants, producing a browning of cauliflower and broccoli heads as well as a spotting of leaves and pods. These symptoms have been described in detail. The fact that the two organisms are carried on and in the seed and may cause serious loss by means of damping-off, a wire-stem, and cankers on the stems and cotyledons of seedlings has not been generally recognized in the literature. The present work was undertaken to verify former statements regarding pathogenicity, environmental factors, and means of control, especially as they concern the production of plants in the seed-bed.

Alternaria herculea was once named A. brassicae (Berk.) Bolle,³ and much confusion exists in the literature regarding the actual organism under discussion, since the writers often fail to give the author's name of the species to which they are referring. A. brassicae (Berk.) Sacc. and A. herculea (Ell. & Mart.) Elliott, however, can readily be distinguished by morphological and cultural characters.

Alternaria brassicae (Berk.) Sacc. is the species with small dark spores. It grows rapidly on potato-dextrose agar, forming colonies at first dark olivegreen, then becoming almost black when the color of the conidia changes to brown. It sporulates abundantly and may produce long chains of more than ten conidia. It does not cause chromogenic reaction on rice medium. The spores germinate over a wide range of temperature, no sharp optimum temperature for germination being noticeable (Fig. 1).

Alternaria herculea (Ell. & Mart.) Elliott bears large light-brown spores. The cultures on potato-dextrose agar are white at first, then become brown when the conidia are formed, and on rice medium a light brown chromogenic reaction is produced. The fungus sporulates very poorly. The spores are sensitive to temperature variations; the optimum temperature for germination is somewhere between 17.2° and 21.1° C. Below and above these limits germination drops sharply (Fig. 2).

¹ An abridgement of a dissertation submitted to the Faculty of the Graduate School of Cornell University, August, 1944, as a major thesis in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology.

² Plant Pathologist in the Ministry of Agriculture, Brazil. Under a fellowship of the Institute of International Education, later with a prize of travel awarded by the Brazilian Government.

The author wishes to express his sincere appreciation to Drs. C. Chupp and L. J. Tyler for their helpful suggestions and criticisms.

³ Bolle, P. C. Die durch Schwärzepilze (Phaeodictyae) erzeugten Pflanzenkrankheiten. Med. Phyt. Lab. Willie Commelin Scholten, Baarn. 7: 1–77. 1924.

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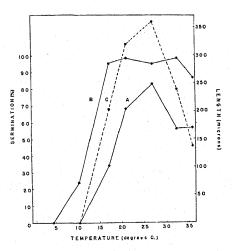


FIG. 1. Relation of temperature to rate of germination of conidia of Alternaria brassicae from a 17-day-old culture, after 9 hours (A), and 18 hours (B); and length of germ-tubes after 13 hours (C).

When flowers are inoculated with Alternaria brassicae, small, brown, ill-defined, and elongated lesions on the petals are produced. Minute, circular, brown spots appear on the flower pedicels and calyces. On the inoculated pods the lesions are small, purple-brown on green pods, or dark brown on dry pods. The young pods may be distorted, cease to grow, and the affected pods dry out from the tip and shatter prematurely. The seeds themselves do not have definite lesions, but seeds in infected young pods do not develop fully, and those in infected, maturing pods are shriveled and non-viable.

The cotyledons of seedlings developed from infested or infected seeds exhibit sharply delimited dark-brown to black spots, usually circular, and more or less sunken (Fig. 3, a). These spots appear more frequently on the

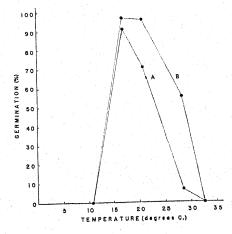


Fig. 2. Relation of temperature to rate of germination of conidia of Alternaria herculea from a 20-day-old culture, after 8 hours (A), and 13 hours (B).

dorsal side and their location seems to be related to the folding of the cotyledons in the seed (Fig. 3).

Damping-off may occur when very young seedlings are affected. The spots on the hypocotyl of young seedlings are narrow, dark-brown to black and about one millimeter long (Fig. 3, b). The base of the stem has a brown, soft, hydrotic area, and later the plant bends over and dies. When infection occurs after the tissues have become harder, the seedlings are not killed, but the cortical cells at the base of the stem collapse, forming a dark, thin layer of dead cells. Because of this withering the diameter of the stem below the cotyledonal leaf scars is reduced. As the central cylinder is not affected, the

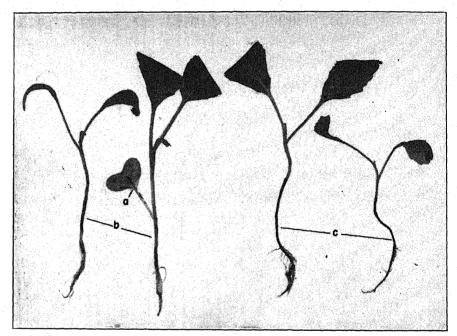


Fig. 3. Spotting of the cotyledon (a), and two phases of the damping-off stage (b and c) on seedlings of cabbage grown from inoculated seed sown in steam-sterilized soil.

plants can survive, but the stems become distorted and the growth of the plant is impaired (Fig. 3, c). Such symptoms are similar to those of wirestem caused by *Rhizoctonia solani* Kühn. Pre-emergence damping-off may occur.

When surface-inoculated cabbage seeds were sown in sterilized soil in greenhouse flats there was 48.3 per cent of pre-emergence damping-off; and of the plants remaining, 51.7 per cent had post-emergence damping-off, and 38.4 per cent had wire-stem within twenty-five days.

Inoculation experiments were conducted in the greenhouse for the studies on pathogenicity. Conidia of *Alternaria brassicae* produced on potato-dextrose agar were suspended in 1–500 gelatin solution and atomized on the

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plants, which were then placed in a moist chamber for approximately 48 hours. Plants then were transferred to greenhouse benches. The gelatin was used to improve the wetting ability of the spore suspension, resulting in a more uniform deposition of the inoculum, and it eliminated the necessity of rubbing the leaves, as practiced by previous workers.

The inoculation experiments demonstrated that Alternaria brassicae (as well as A. herculea) is a virulent pathogen and that injury is not needed for infection. The somewhat general belief that the fungus attacks preferably the older leaves seems not to be sound. A better explanation for the fact that mostly the lower leaves (which are the older) are infected is that the organism sporulates on decaying parts in contact with the soil and the spores are splashed by the rain drops over the lower leaves. Sometimes these leaves remain in contact with the soil and are likely to become infected because of the proximity of conidia formed on the debris.

The minimum period of wetting required for leaf infection was also investigated. Inoculated cabbage plants were incubated in a moist chamber and groups of them were removed at intervals of six hours. The temperatures ranged from 19° to 28° C. Infection was not evident in plants removed after 6, 12, and 18 hours, whereas those remaining in the moist chamber for 24 hours or longer were infected.

Examination of several seed samples revealed that frequently spores are carried on the surface of the seed and that these spores may remain viable for a long time. Spores obtained from seed samples about twenty months old and stored at 32° F. for fourteen months showed high germinability. When planted on plain agar several of the seeds were covered with mycelium and spores, and the growth of the seedlings attacked by the fungus was checked. When seeds were sown in pots, characteristic symptoms of the disease were produced on the seedlings.

There is good evidence that the fungus sometimes is carried as latent mycelium in the seed. Growth of Alternaria brassicae developed from naturally infected seeds of kale subjected to each of the following treatments: (a) hot water at 52° C. for 20 minutes; (b) seeds dipped in 95 per cent alcohol, treated with corrosive sublimate 1–1000 for 5 minutes, and washed with four changes of sterilized distilled water; (c) seeds dipped in 95 per cent alcohol, treated with corrosive sublimate 1–1000 for 5 minutes, and washed with two changes of Clorox 25 per cent; (d) seeds treated with Clorox 25 per cent for 5 minutes and dried on filter paper.

Tests were made to check the effect of hot water and of corrosive sublimate on spores. Spores of 12-day-old cultures suspended in water were held at 52° C. for 5 minutes in a water bath. In another experiment, spores of a 12-day-old culture were suspended in corrosive sublimate 1–1000 for 3 minutes, caught on filter paper, and then washed with four changes of sterilized distilled water. These treated spores when placed in 1 per cent sugar solution did not germinate within 24 hours, whereas 97 per cent of the untreated spores germinated.

These facts lead to the conclusion that a latent mycelium must be present inside the seeds, as it does not seem possible that the spores on the surface of the seed could stand the treatments that were given.

In other experiments, seeds of bok-toy and radish were dipped in 95 per cent alcohol, treated with corrosive sublimate 1–1000 for 5 minutes, washed with three changes of sterilized distilled water and planted on potato-dextrose agar. Within five days a white mycelium grew from a few seeds, and spores of Alternaria herculea were found in microscopic mounts from these cultures. A hyaline mycelium was present in the epidermis of seeds of the same samples. Sections of radish seeds contained a hyaline, septate, branched mycelium, 4 microns wide, that extended into the endosperm layer. These observations support the statement that this pathogen can be carried underneath the seed coat.

Since Alternaria brassicae sporulates abundantly on decaying vegetable matter, the soil may be an important harborer of the pathogen. Thus, the pathogen can possibly be carried from the seedling beds to the field in soil adhering to the seedling roots, or on seedlings that have been splashed by rain or overhead irrigation. It also is possible for the seedling to have infections in an incipient stage that later result in the killing of the plant or part of it, and consequent production of spores in the field. In this manner, the seedlings grown in infested seedling beds may be of great importance in the dissemination of the inoculum.

The longevity of spores was tested by exposing cultures of the fungus on potato-dextrose agar to outdoor weather during January to July, in which the organism was exposed to temperatures ranging from -23° to $+30^{\circ}$ C. At the end of this period the spores were still viable and pathogenic, as shown by their germination on slides and their ability to induce disease in inoculated cabbage plants. These results, however, do not warrant final conclusions regarding the behavior of the organism in the soil.

The effectiveness of chemicals in seed treatment for the control of Alternaria brassicae was studied in the greenhouse. For these studies, Alternaria-free cabbage seed was inoculated with spores suspended in a gelatin solution (1–500), dried, and treated. The dosages of the chemical materials applied as dusts were dependent upon the amount of chemical adhering to the seeds. Aside from the dust treatments, some seeds were soaked in a 1–1000 solution of corrosive sublimate for 5 minutes, and dried. Inoculated and non-inoculated untreated seeds were included to check the germination of the seeds. Four replications of 100 seeds for each treatment were planted in flats in steam-sterilized soil. Pre-emergence damping-off was estimated by assuming that the differences in stand between the non-inoculated check and the treatments were due to the pathogen. Table 1 gives the total number of seedlings affected for each treatment, after 25 days.

Semesan and Arasan were effective in reducing the amount of damping-off and wire-stem. While Wettable Spergon checked the pre-emergence damping-off, it was not efficient against post-emergence damping-off. The

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TABLE 1.—Results of seed treatments for the control of Alternaria brassicae on surface-inoculated cabbage seeds

				Damping-off				
Treatment		otal gence	Final stand	Pre- emergence (estimate)	Post- emergence ^a		Wire stem ^b	
Arasan	2	283	278	9	5		4	
Semesan	2	289	285	3	4	1	9	
Fermate	5	273	247	19	26		23	
Wettable 604	2	272	269	20	3		6	
Wettable Spergon	9	284	263	8	21		5	
Corrosive sublimate		269	218	23	51		13	
Check (inoculated)		151	73	141	78		28	
Check (not inoculated)		292	290	0	0		0	

a Post-emergence damping-off:

Least difference for odds of 19:1=31.5 seedlings

b Wire stem:

Least difference for odds of 19:1=14.5 seedlings

reverse was shown by Wettable 604. The other two treatments did not control damping-off.

SUMMARY

Alternaria brassicae (Berk.) Sacc. that causes a leaf spot, pod spot, and general browning of heads of cauliflower and broccoli and other cruciferous plants is also the cause of damping-off, wire-stem, and spotting of seedlings. This pathogen is readily distinguished by morphological and cultural characters from A. herculea (Ell. & Mart.) Elliott which likewise causes a disease of cruciferous plants.

Both Alternaria species are virulent pathogens and are able to infect the suscept at any age and independently of any injury. A period of wetting for at least 18 hours is necessary for infection.

The seeds may carry both pathogens as spores or as latent mycelium in the seed.

The spores of Alternaria brassicae may retain their viability and their pathogenicity for more than six months. Seedlings grown in infested seed beds may carry the inoculum to the field. In the field the sources of inoculum are the dead lesions and the decaying plant parts on the soil. Water is the main agent of dissemination.

Semesan and Arasan were effective in reducing the amount of damping-off and wire-stem on seedlings originated from surface inoculated seeds.

DEPARTMENT OF PLANT PATHOLOGY.

CORNELL UNIVERSITY.

ITHACA, NEW YORK.

INOCULATION EXPERIMENTS WITH PSEUDOMONAS RIBICOLA

G. W. BOHN AND J. C. MALOIT¹

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INTRODUCTION

The first pathogenicity test of $Psudomonas\ ribicola$, which causes a defoliating spot disease of $Ribes\ aureum\ Pursh$. described by Bohn and Maloit (1), was made in the field at Cheyenne, Wyoming, in July, 1940. Both surfaces of leaves on vigorous shoots were sprayed with $\frac{1}{10}$ dilutions of 48-hour beef-extract broth cultures before, during, and immediately after a thundershower between 6 p.m. and 8 p.m. Each shoot in 1 series was wrapped in moistened cheesecloth immediately after spraying; each shoot in a second series was enclosed in a large, brown paper bag. In spite of these seemingly excellent conditions for infection, no leaf spots occurred on the 400 or more leaves on 24 shoots inoculated. Because the 6 bacterial isolates used were typical of numerous isolates obtained by approved methods, and because bacteria were found in all spots of the type under study that were examined with the microscope in 1939 and 1940, other methods of inoculation were tried.

In January, 1941, thirty plants in 2-gallon stone jars were pre-treated for 24 hours in a greenhouse moist chamber. Three check plants were sprayed with sterile broth and 1 young leaf on each plant was pricked slightly 10 to 12 times with a sterilized needle immediately after spraying. Each remaining lot of 3 plants was inoculated with 1 of 9 different bacterial isolates. Each plant was replaced in the moist chamber immediately after inoculation. No spots occurred on the checks; twelve spots resulted from the spray inoculations (approximately 600 leaves); 31 spots, from the needle punctures (approximately 300 punctures). The few infected spots that resulted from spray application of bacterial suspensions may have occurred in wounds caused by insects although very few insects (white flies) were present on these plants. The low proportion of infected spots resulting from needle punctures and the occurrence of holes in the centers of the spots suggested that some other method causing smaller wounds might yield infection spots more abundant and more comparable with those resulting from natural infection.

LITERATURE

A review of the general literature revealed a paucity of methods of inoculation recommended for bacterial plant pathogens. This is especially true with diseases that do not become systemic, infections being limited to local lesions on leaves, tender stems, and fruits (2, 3, 6, 7, 11, 12, 13, 14). The

¹ Associate Plant Pathologist and formerly Agent, respectively, Cheyenne Horticultural Field Station, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture.

methods generally recommended are based on modes of ingress of bacteria into plant tissues in natural infections. Spraying has been recommended for those bacteria that gain access to susceptible tissues through stomata or directly through hydathodes, lenticels, nectaries, or other noncutinized Treatment of host plants in moist chambers before and after spraying has been recommended to keep the stomata open, and to increase moisture present in intercellular spaces and on outer surfaces. spraying with water before application of the bacterial suspension and forced spraying with bacterial suspensions have been used to aid bacteria to penetrate stomatal openings. Wounding with a needle or similar tool has been recommended for those bacteria that gain access to susceptible tissues through wounds caused by insects, hail, or other agents. been recommended that with any particular disease under study, inoculation methods should imitate natural infection methods under comparable conditions of the host and environment. Therefore, observations on natural infections of plants in the field were reviewed.

NATURAL INFECTION OF GOLDEN CURRANT BY PSEUDOMONAS RIBICOLA

At Cheyenne, Wyoming, bacterial spots appear on currants in the middle of June. The spots are most abundant on the first leaves to grow in the spring and the general absence of distortion suggests that infection occurs after the leaves have nearly matured. Therefore, natural infection must occur in late May or early June. The weather at Cheyenne during this part of the spring is cool, cloudy, and windy. The maximum daily temperature usually is between 65° and 80° F. (19° to 26° C.); the minimum, between 35° and 50° F. (1° to 10° C.). Although total rainfall is slight. the sky usually is cloudy, the relative humidity is high on frequent occasions, and thundershowers, often accompanied by hail or sleet, are frequent. The average wind velocity for May and June usually is 9 to 12 miles an hour, and high winds or gales frequently occur. These observations on natural infection and the weather data suggest that natural infection by Pseudomonas ribicola in currants occurs in minute wounds resulting from windblown hail, sleet, or sand or from buffeting of leaves against other leaves and branches. The observations suggest also that infection is favored by low temperature and high relative humidity. These conclusions are supported by the occurrence of necrotic bacterial lesions in hail-injured spots on young shoots and by the continued enlargement of spots in detached leaves placed in a moist chamber in the refrigerator at approximately 3° C.

COMPARISON OF INOCULATION TECHNIQUES WITH BACTERIAL SPOT OF CURRANT

Three techniques of inoculation that might yield results with currants more comparable to natural infection than do those usually recommended for use with bacteria were suggested by the virus infection techniques described by Holmes (4), Jones (5), Rawlins and Tompkins (8, 9, 10), and

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n -II -II S Riker, Ark, Elliot, Hildebrand, and Leach (12). These methods are (a) Bacterial suspensions from agar cultures can be stippled on leaves supported by fingers or wooden pot labels with previously disinfected (with 70 per cent alcohol) lacquer brushes that have short, moderately stiff bristles; (b) Bacterial suspensions can be rubbed on leaves with previously sterilized fingers (2) or cotton or cheesecloth swabs (4); (c) Bacterial suspensions can be rubbed on leaves (as in b) dusted with carborundum (8, 9).

These methods were compared with spraying and needle-wounding methods using *Pseudomonas ribicola* on leaves of *Ribes aureum* grown in pots in the greenhouse during the winter of 1940–41. As all of the repeated experiments yielded similar results, only one, which included all of the methods compared at this time, is reported in detail.

Two-year-old currant seedlings were transplanted from the field nursery to 3-inch pots and forced into growth in the greenhouse. Vigorous seedlings were selected for inoculation. Following inoculation, the plants, with the exception of 1 group dusted with carborundum, were placed under bell jars in the laboratory at approximately 21° C. for 48 hours, then incubated in a cool greenhouse (day 18° to 25° C., night 8° to 15° C.) for 2 weeks. Tap water suspensions of Pseudomonas ribicola from 4-day-old beef-extract-dextrose-agar cultures were used as inoculum. Instruments and hands were disinfected in 70 per cent alcohol and then washed in tap water between treatments. Cotton, cloth, and pot labels were heated in glass containers in an electric oven at 170° to 180° C. for 2 hours before use. Five treatments were compared.

- 1. Suspensions were brushed with lacquer brushes on several leaves of each of 5 plants. Check leaves were brushed with tap water.
- 2. Suspensions were sprayed on the leaves of 5 plants. Check plants were sprayed with water.
- 3. Suspensions were rubbed on several leaves of each of 5 plants with coarse cheesecloth swabs. Check leaves were rubbed with a swab wet with water.
- 4. Suspensions were rubbed on several leaves of each of 5 plants with coarse cheesecloth swabs after the leaves had been dusted with 300-mesh carborundum. Check leaves were dusted with carborundum and rubbed with a swab wet with water.
- 5. Three plants were inoculated as in treatment 4, but left uncovered in the greenhouse immediately after inoculation (i.e., they were not put in a moist chamber).

After 2 weeks' incubation numerous typical bacterial spots (15 to 50 on a leaf) had developed on leaves inoculated by brushing (Treatment 1) and on those inoculated by rubbing carborundum-dusted leaves (Treatments 4 and 5); no difference could be noticed between carborundum-dusted inoculated plants incubated 48 hours under bell jars in the laboratory and those left uncovered in the greenhouse for the entire incubation period. Fewer spots (2 to 13 on a leaf) had developed on leaves rubbed without carborundum (Treatment 3), and only 1 spot occurred on the sprayed leaves.

No effects of brushing check leaves could be seen with the unaided eye; but slightly raised streaks, not different in color from the rest of the leaf, were visible at $10 \times$ magnification or greater. It is possible that injury was limited to removal of cuticle, but some epidermal cells may have been injured. This type of injury probably occurs commonly in the field at Cheyenne as leaves are brushed against each other and against stems by the wind. A few small brown flecks, barely visible to the unaided eye, occurred on carborundum-dusted leaves rubbed with a cheesecloth swab. Injury not visible to the unaided eye obviously occurred also (9). This type of injury would be expected to occur commonly at Cheyenne from wind-driven sand and sleet. No injury was observed on leaves sprayed or on those rubbed without carborundum.

Additional inoculation experiments were conducted during the winter of 1943–44. In two series of tests, water suspensions of *Pseudomonas ribicola* from potato-beef-extract-sucrose-agar cultures were used on young leaves of *Ribes aureum* clones grown in 2-gallon stone jars in the greenhouse. The plants were incubated in a greenhouse moist chamber at 15° to 25° C. for 24 hours after inoculation; then removed to a cool greenhouse (18° to 25° C. day and 8° to 15° C. night) for incubation. All implements were disinfected in 70 per cent alcohol or by heat before use.

Three treatments were compared in the first series.

- 1. The suspension was sprayed on both surfaces of the leaves of 4 plants; checks were sprayed with sterile water.
- 2. The leaves of 4 plants were dusted with 300-mesh carborundum and sprayed with the suspension; checks were dusted and sprayed with sterile water.
- 3. The leaves of 10 plants were dusted with 300-mesh carborundum and rubbed with a cotton swab; checks were dusted and swabbed with a sterile moist pad.

No injury or infection resulted from the simple spray treatment. All leaves dusted with carborundum and sprayed with bacterial suspensions developed moderate numbers (2 to 15) of typical bacterial spots; checks showed no injury. All leaves dusted with carborundum and rubbed with a cotton swab dipped in bacterial suspensions developed numerous (30 to 80) typical spots. Check leaves dusted with carborundum and rubbed with sterile pads developed a few minute brown flecks which apparently resulted from mechanical injury.

In the second series of tests, 7 treatments were compared on currant plants kept in a greenhouse moist chamber for 15 hours before and 24 hours after treatment and incubated for two weeks in a cool greenhouse.

- 1. A moderately turbid bacterial suspension was sprayed on both surfaces of the leaves. Checks were sprayed with sterile water.
- 2. The bacterial suspension, with 300-mesh carborundum added, was sprayed on both surfaces of the leaves. Checks were sprayed with a sterile carborundum suspension.

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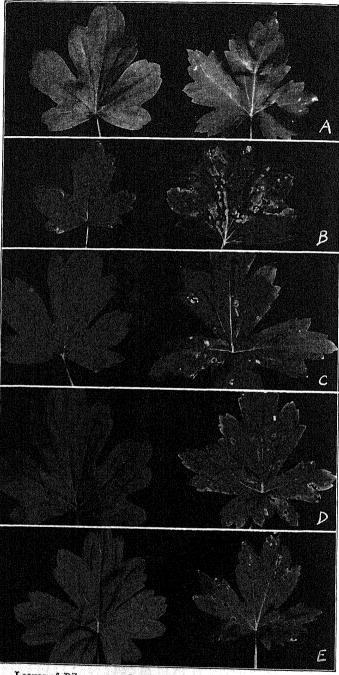


Fig. 1. Leaves of Ribes aureum inoculated with suspensions of Pseudomonas ribicola. Greenhouse, Cheyenne, Wyoming, 1944. Checks at left, inoculated leaves at right. A, Spray; B, Forced spray; C, Cotton swab; D, Carborundum dust and cotton swab; E, Needle.

- 3. A $\frac{1}{10}$ dilution of the suspension used in treatment 1 was sprayed against leaves with sufficient force to watersoak small regions of leaf tissue. Checks were sprayed similarly with sterile water.
- 4. Needles were dipped in a moistened agar culture and used to stipple leaves. Checks were stippled with sterile needles.
- 5. Needles were dipped in a moistened agar culture and drawn across leaves with light pressure insufficient to cause obvious wounds. Checks were similarly scratched with sterile needles.
- 6. Leaves were rubbed with a cotton pad moistened with the bacterial suspension used in treatment 1. Check leaves were rubbed with a cotton pad moistened with sterile water.

TABLE 1.—Effectiveness of different methods of inoculating leaves of golden current with Pseudomonas ribicola in the greenhouse at Cheyenne, Wyoming, March, 1944

		Plants	Spots	s on	Visible	Q		
Method		treated, excepting checks	Young leaves	Mature leaves	injury on checks	Comparison with natural infection		
		Number						
1.	Spray	3	Rare	None	None	Spots typical		
2.	Spray, car- borundum	3	Moderate	Rare	do	do		
3.	Forced spray	y 3	Abundant	Abundant	do	Severe killing of large areas and extreme distortion rarely seen in natural infections		
4.	Needle puncture	3	On 75 per cent of punctures	On 10 per cent of punctures	Holes	Similar holes rare in spots resulting from natural infections		
5.	Needle scratch	3	On 75 per cent of scratches	On 10 per cent of scratches	Scratches, distortion	Similar mechanical in- jury not abundant in natural infections		
6.	Cotton swab	5	Moderate	None	None	Spots typical		
7.	Carborundur dust and cotton swa		Abundant	Rare	Slight flecks	Spots typical. Com- parable flecks would probably be over- looked in natural in- fections		

7. Leaves were dusted with 300-mesh carborundum and rubbed with a cotton pad moistened with the bacterial suspension used in treatment 1. Check leaves were dusted with carborundum and rubbed with a cotton pad moistened with sterile water.

The data on infection and mechanical injury, together with comments on comparisons with natural infections are presented in table 1 and figure 1. It is apparent that *Pseudomonas ribicola* is a wound parasite and rarely enters currant leaves through stomata. The best of these seven techniques of inoculation was that in which leaves were dusted with carborundum and rubbed with a pad moistened with a suspension of the bacteria. Other methods either resulted in few foci of infection or caused injury not comparable to injury observed in naturally infected currants in the field.

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DISCUSSION

The abundant infections that resulted from applications of suspensions of Pseudomonas ribicola to wounds in leaves of Ribes aureum demonstrated that this bacterium is the pathogen associated with a necrotic spot disease of the golden currant. The general failure to obtain infections in leaves on plants kept in moist chambers and sprayed with suspensions of this organism indicated that the bacterium is a wound parasite and usually does not enter leaves through stomata. Observations on the abundance and appearance of infections that resulted from applications of suspensions by different methods, together with observations on natural infections and the weather, indicated that Ps. ribicola is a wound parasite which usually enters currants through minute wounds caused by wind-driven sand and sleet and buffeting of plant organs against each other in the wind.

The abundant typical infections by Pseudomonas ribicola in currant leaves obtained with 2 inoculation techniques, new or little described for use with bacterial plant pathogens, suggest that these methods may have value in studies on other bacterial diseases of plants. The techniques are (1) application of bacterial suspensions with lacquer brushes or other kinds that have moderately stiff bristles, and (2) application of bacterial suspensions with cotton or cloth pads to leaves or other organs dusted with carborundum (Rawlins and Tompkins' carborundum technique for virus inoculation). These techniques have other characteristics that suggest their probable utility in plant breeding programs concerned with resistance to diseases caused by bacteria and fungi. Inoculations can be made with comparative ease and rapidity and the symptoms, at least those of bacterial spot of currant, are comparable to symptoms that result from natural infections. Numerous infections result when these techniques are used on plants placed on open greenhouse benches, i.e., without the use of moist chambers. These results suggest that ingress of bacteria into susceptible tissues occurs at the time of inoculation. This agrees with the observations by Holmes (4) on pad inoculations with viruliferous plant extracts. If this is true, the brush and the carborundum techniques may have value in large-scale inoculation experiments with plant pathogenic bacteria and fungi, especially those dependent on wounds for ingress. They should be particularly useful with hosts that have hard, thickly cutinized leaves, and in field inoculations where control of relative humidity is difficult.

A sample of 600-mesh carborundum was unsatisfactory in these experiments, while a sample of 300-mesh carborundum yielded excellent results. Microscopic examination revealed few angular particles in the 600-mesh sample; most of the particles were smooth and nearly spherical. The 300-mesh sample consisted nearly entirely of angular, sharp-edged particles with dimensions varying from 5 to 20 microns. These data and the discussion of carborundum samples by Rawlins and Tompkins (10) indicate that one should be able to predict the value of a sample for use in inoculation work

by microscopical examination. Although 300-mesh dust was used in all experiments reported here, other sizes may be found more satisfactory for use with hosts other than currant.

The atypical symptoms caused by watersoaking leaf tissue with bacterial suspensions was further indicated in experiments with other plants. Regions of leaves of tomato, raspberry, and strawberry watersoaked by forced spraying with suspensions of Pseudomonas ribicola were killed within 3 to 5 days although sterile water similarly forced into them caused little or no injury. Repeated inoculations by other methods, including the carborundum dust and cotton pad technique, failed to yield infection in these plants. Apparently, necrosis following forced spray applications severe enough to cause saturation of leaf tissues with bacterial suspensions is not a reliable test of pathogenicity. This does not apply, of course, to the spray method in which leaves are watersoaked with sterile water before application of bacterial suspensions to the surface. Leaf tissues usually are little injured by watersoaking with sterile water, although occasionally injury has occurred in tomato leaves so treated. The results obtained with forced sprays on immune plants indicate that the use of sterile water as a check for inoculations with bacterial suspensions may not be a reliable check. It would seem advisable to use suspensions of killed bacteria or suspensions of other species of bacteria not pathogenic to the host as checks in critical studies on parasitism.

SUMMARY

Methods of inoculation generally recommended for use with bacterial plant pathogens resulted in little infection by *Pseudomonas ribicola* in currant leaves or in symptoms unlike those resulting from natural infections. Inoculations with moderately stiff brushes and with cotton pads rubbed on leaves dusted with 300-mesh carborundum yielded abundant infections which produced symptoms comparable to those resulting from natural infections. Intermediate numbers of infections resulted from spray applications of bacterial suspensions on carborundum-dusted leaves. These results, together with observations on natural infections and the weather, indicated that *Pseudomonas ribicola* is a wound parasite which naturally enters currant leaves through minute wounds caused by buffeting of leaves against other leaves and branches in the wind and by wind-driven sand and sleet.

The probable value of these techniques in programs on breeding crop plants for disease-resistance is suggested. The brush and the carborundum dust and cotton pad techniques should prove successful with bacteria that usually invade hosts through minute wounds, especially with hosts that have hard, thickly cutinized surfaces.

HORTICULTURAL FIELD STATION,
CHEYENNE, WYOMING,
U. S. DEPARTMENT OF AGRICULTURE.

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INFECTION EXPERIMENTS WITH DETACHED WATER-CONGESTED LEAVES¹

JAMES JOHNSON

(Accepted for publication August 3, 1945)

The relation of water congestion (4) in plant tissues to disease predisposition and to varietal susceptibility is being investigated through a number of different means in this laboratory. One of the more simple and promising methods of study has involved the use of detached leaves of plants treated in various manners before, during, and after inoculation with a variety of parasitic and saprophytic organisms, viruses, toxins, chemicals, and inert substances such as India ink and dyes. The use of detached leaves for infection experiments is not new, but in combination with prior water congestion introduces additional highly favorable conditions for infection. The detached-leaf method permits ready comparisons of tests under a fairly uniform set of conditions which are not otherwise easily obtained or maintained. A number of tests, for example, may be conducted on a single leaf when using such large leaves as those of tobacco. Only a brief summary of the results of these studies need be presented to suggest its applicability to further studies and to show the important relationship of water congestion in leaf tissues to the mechanism of infection and the further development of disease. Earlier papers from this laboratory have dealt with some of the more general aspects of the subject (3). The problem is related to, but not identical with, the more frequently recognized phenomenon of "storm water-soaking" as described by Clayton (1).

MATERIALS AND METHODS

Detached, disease-free leaves of many plants, including tobacco, may be maintained in approximately normal condition in covered containers for periods of 6 to 12 days at room temperatures. Using sterilized refrigerator pans $(12 \times 7 \times 4 \text{ inches})$ for large individual leaves and for different treatments, fairly satisfactory conditions for infection on such living tissue cultures may be obtained. The absence of light and the high humidity provided by moistened, paper toweling on the bottom of the pan seem to favor the prolongation of the life of the detached leaf while at the same time providing suitable conditions for disease development. Removing the covers momentarily 2 or 3 times daily supplies sufficient aeration. Water-congested leaves decline faster than uncongested leaves, but the congestion need not be allowed to continue for more than 18–24 hours at the maximum. Tobacco leaves appear to be well adapted to this technique; whereas potato leaves, for example, apparently do not tolerate such methods of treatment. A great

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¹ Approved for publication by the Director of the Wisconsin Agricultural Experiment Station and by the Division of Tobacco Investigations, United States Department of Agriculture.

variety of plant species has been used sufficiently to show adaptability over a wide range of hosts.

Strains of the variety of tobacco known as Havana-Seed were used mostly in the present experiments. For the virus tests, the hybrid host ($Nicotiana\ tabacum \times N.\ glutinosa$) was used. This plant yields good local lesions within 3 days with the ordinary tobacco-mosaic virus.

The detached leaves were congested individually by applying high water pressure to the cut end of the leaf midrib or petiole in equipment as previously described (3). Each leaf usually required 5 to 30 minutes' treatment under water pressure to effect water congestion of one-half or more of the leaf area (Fig. 1, C, D). The congestion may disappear after 1 to 3 hours of drying or it may be maintained up to 24 hours in the containers without seriously affecting the subsequent condition of the leaf. The time that the water congestion is allowed to remain in the leaf may be regarded as the incubation period, although it may often overlap with both long penetration periods or short periods required for disease development.

The inoculum used in most of the trials was a beef-broth-peptone suspension of the tobacco wildfire organism [Phytomonas (Bacterium) tabaci (Wolf and Foster) Bergey et al.]. One advantage of using this organism was its ability to produce a typical toxin in culture, which could easily be studied separately by merely killing the bacteria in the culture by sterilization, leaving the toxin active. When a culture of the wildfire organism is used at high concentrations (below approximately 1 to 100), it may be difficult to distinguish between the effect of the toxin and that of the organism. The blackfire organism [Phytomonas (Bacterium) angulata (Fromme and Murray) Bergley et al.] provided a closely related bacterial parasite lacking appreciable amounts of toxin, although the wildfire organism can also be freed from the toxin by special methods.

Several fungi have been used in the trials including normal saprophytes such as Aspergillus niger v. Tiegh. and Penicillium spp. Other organisms such as Septoria lycopersici Speg. and Helminthosporium sativum Pam., King and Bakke not parasitic on tobacco, and some typically parasitic only on other organs of tobacco (i.e., Thielaviopsis basicola (Berk. and Br.) Ferr., Pythium sp. and Rhizoctonia sp.) have been inoculated to tobacco leaves. Only one typical fungus parasite of tobacco leaves, namely, Cercospora nicotianae Ell. and Ev. was available for this study.

The virus used in the studies was that of ordinary tobacco mosaic which produces local lesions in 2 to 3 days on detached leaves of the hybrid host used when properly inoculated. The inert substances used most frequently have been India ink, which may be regarded as of a particulate size roughly comparable to bacteria, and water-soluble dyes such as rose bengal, which is of only molecular size as regards the orifices through which it will pass. Eosin and safranin have also been used with good results.

These various materials or inocula may be applied in various ways such as by droplets, immersion in baths, by wiping methods, or by atomizing with

varying forces, and in the presence or absence of different kinds of wounds or positions of stomatal openings. The inoculum may be largely or entirely removed from the leaf surface with running water at any desired time following application, thus in some measure regulating the period of penetration allowed. This has been usually less than 3 minutes (immediate), 1 to 3 hours (slow), and 18–24 hours (very slow). In the case of the organisms, a continued environment favorable for visible expressions of infection and disease were maintained in the refrigerator-pan moist chamber. A considerable part of the experiments dealt with the period that the water-congested leaves were allowed to remain congested following inoculation, but these results, although showing striking influences on the severity of disease, are not pertinent to penetration or original infection.

EXPERIMENTAL RESULTS

A considerable variety of combinations of conditions were usually compared in each individual experiment, which does not permit a convenient and detailed summarization of an extensive amount of data. For present purposes, it appears sufficient to present a summary of the typical results (Table 1). Since all the conditions of the individual trials, in some cases, cannot be constantly controlled (i.e., stomatal position), exceptions to the results presented may be expected in efforts at close replication in some groupings. Considerable variation may be expected, especially between unwounded greenhouse-grown and outdoor-grown leaves. The location of the more irregular or variable behavior is indicated in the table.

Behavior of Normal Leaves

Considering the data largely from the point of view of water congestion in the leaf tissue and the possible avenues of penetration by various substances including different types of organisms, the results with normal (uncongested or control) leaves may be presented first. The normal or control leaves were in all cases tested simultaneously with the other forms of treatment and, except for such treatments, the conditions for penetration, infection, or disease development were approximately identical.

Reference to table 1 shows that none of the substances or organisms used appreciably penetrated uncongested leaves or caused infection. Cercospora nicotianae yielded some signs of infection in a small percentage of inoculations after prolonged incubation periods. If droplets of inoculum are allowed to remain on the leaf for extended periods, it is possible that minute amounts of water may enter the substantial chambers, thus promoting penetration. Although water and dye evidently do not enter from surface droplets on uncongested leaves, the immersion of normal leaves occasionally favors congestion and penetration of substances, thus suggesting a very delicate balance in pressures. In any case, the infection tubes of fungi may presumably penetrate the stomata through their own efforts in the absence of water congestion, which the bacteria are evidently unable

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When a normal leaf is wounded, however, a new set of conditions arises and very different results are secured (Table 1). This is partly due to the removal of the epidermal barrier to penetration and partly to the congestion of the intercellular spaces adjacent to the wound. Even if surface water is not present on the wound, the cell sap released by wounding is often sufficient to cause congestion. When droplets of watersoluble, nontoxic dye or India ink are placed on needle-prick wounds, these

TABLE 1.—Typical results on penetration and infections of detached tobacco leaves treated in various ways with regard to type of water congestion and of wounding and subsequently maintained in moist chambers for several days

Material or inoculum	No water congestion			Internal water congestion		Water congestion by atomizing	
applied	No wounds		Wiping ^b wounds	No wounds	Needle wounds	Slight	Distinct
Dye (rose bengal) India ink Viruse (tobacco mosaic) Toxin (from B. tabacum) Bacteria (B. angulatum) Fungus (C. nicotianae) Fungus (T. basicola)	0 0 0 0 1.7.9	3,7,4 3,7,4 3,7,9 3,7,5 3,4 2,7,9 2,7,9	2,7,4 1,7,4,9 3,6,7 2,7,5 0 0	2,8,4,9 1,8,4,9 2,4,9 2,6,8,9 1,6,8,9 1,5,6,9 1,6,9	3,8,4 3,8,4 3,7,9 3,5,6,8 3,6,8 2,5,6,8 2,6,8	2,4,9 1,4,9 2,4,9 2,5,9 1,6,7,9 1,5,7	3,8,4 3,8,4 3,4,9 3,6,8 3,6,8 2,5,6,8 2,6,8

a 0, No penetration.

Very slow penetration (18-24 hrs.).

Slow penetration (1-3 hrs.).

Immediate penetration (1-3 min.).

No further progress.

Chlorosis.

Necrosis.

Small area or incipient infection.

Large area or heavily diseased.

Irregular behavior, depending upon stomatal openings, degree of wounding, etc.

b With cloth and carborundum.

c No visible permanent injury to cuticle. d Some lasting injury to cuticle on uninoculated area.

o On hybrid host.

inert substances enter the congested area at once, as do bacteria or other particles of sufficiently small size to enter the intercellular spaces (Fig. 1, A). Large spores or mycelium may become only lodged in the wound itself, but infection tubes may subsequently enter the congested area if the condition is maintained for a sufficient time to permit their growth. further progress of wound infections may naturally be checked quickly in leaves if the water supply in the area is lowered to normal amounts, or below, by evaporation. Frequently, little or no progress is made even by virulent parasites in wounded areas of otherwise uncongested leaves maintained under favorable environment for disease. In the instance of organisms producing toxins, chlorotic areas may be conspicuous in the infected area in the presence of very little necrosis.

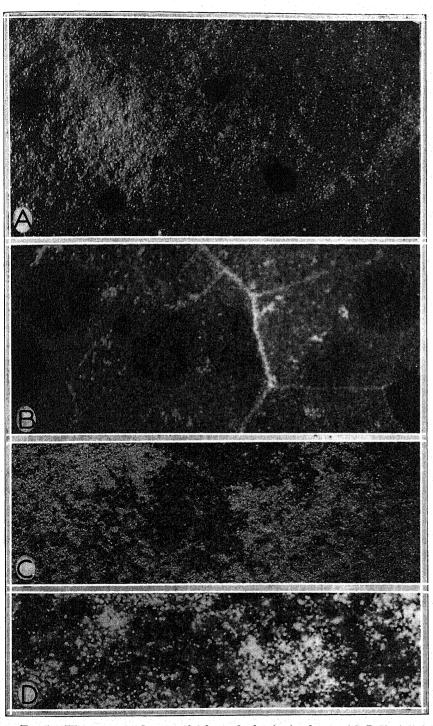


Fig. 1. Water-congested areas of tobacco leaf. A. As shown with India ink following wounding with tiny needle pricks $(\times 7)$. B. India ink propelled into leaf by atomizing $(\times 7)$. C. Water congestion induced by internal water pressures, photographed without staining $(\times 7)$. D. An area similar to C greatly enlarged.

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If the normal leaf is wounded by the "wiping" method, as used in plant virus inoculation, especially in the presence of an abrasive such as carborundum powder, only the plant hairs may be broken or the epidermal cuticle and cell walls scratched. Water congestion does not develop in intercellular spaces, and India ink or organisms do not enter into the intercellular spaces. However, particles of molecular size such as dyes, toxins, and viruses may enter cells through the broken leaf hairs or epidermal cell walls, resulting in coloration, toxic action, or virus infection, respectively. This phenomenon is rather remarkable and unique with the tobacco-mosaic virus, since water from external sources apparently is not necessary for penetration and infection to occur. Infection has been secured with this virus by wiping the leaf surface with dry and aged powder made from mosaic-infected leaves. It seems likely, because of immediate virus penetration into slightly wounded cells, that some force similar to that in watercongested, intercellular spaces is concerned in this form of invasion. Infection with bacteria and fungi is evidently not obtainable by the wiping method, although it is admittedly difficult to perform it without some injury which may permit invasion into intercellular spaces.

Behavior of Internally Water-congested Tissues

When detached leaves are artificially water congested by internal water pressure for experimental purposes, and inoculations made in an identical manner to that of the uncongested or normal leaves, more favorable conditions for penetration develop in the unwounded tissue and a highly intensified type of result occurs adjacent to the wounded tissues.

Penetration through the stomatal openings in the congested, unwounded leaves is of chief interest in relation to host predisposition and disease resistance. It is not certain that more stomata are open because of the water congestion, although it may be assumed that the stomata tend to open in the presence of excess water in the intercellular spaces. It is readily demonstrable, however, that dyes, India ink, and bacterial pathogens enter the intercellular spaces of congested leaves through stomatal openings whereas little or no such entrance occurs in uncongested leaf tissues. When the entire congested leaf is immersed in dye or ink, it is often apparent nevertheless that only a small fraction of the stomata are actually penetrated (Fig. 2, A and B). By the immersion method, too, it is obvious that the penetration is often localized in certain areas of the leaf, that it is influenced by the prior treatment or exposure of the leaf with respect to nutrition, environment, etc., also that it varies greatly with the plant species. Entrance of India ink through the stomata is relatively slow as compared to entrance through wounds; no doubt a consequence of the relatively small orifice through which the material must pass.

It is of interest to note that although the virus of tobacco mosaic can be shown to enter the intercellular spaces for considerable distances, no multiplication or disease results unless some cells are only slightly wounded in some such way as to permit entrance into living cells. The wildfire toxin appears, on the other hand, to enter the unwounded cells from the intercellular spaces, resulting in gradual destruction of the chlorophyll and consequent chlorosis of the affected tissues. The action of the tobacco wildfire organism and certain other bacteria in the intercellular spaces is rapid in water-congested tissue, often producing severe necrosis and collapse of the tissue in 24–36 hours (Fig. 3, D). The signs of infection with fungi may be considerably slower in developing than with bacteria in congested leaftissue, possibly because invasion must be made by progressive mycelial growth rather than direct bodily penetration. Under some conditions with other

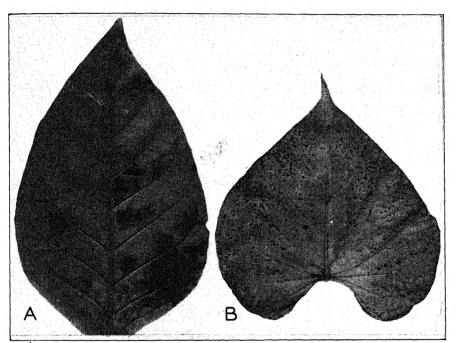


Fig. 2. Stomatal penetration in internally water-congested leaves. A. Tobacco immersed in India ink. B. Morning Glory immersed in rose bengal for about 5 minutes.

hosts and fungus parasites (e.g., Helminthosporium sativum on barley), symptoms of infection have been secured in congested leaves quite as rapidly as with bacterial parasites. With the fungus saprophytes used (e.g., Aspergillus, Penicillium), infection is slow and uncertain. However, Thielaviopsis basicola, typically parasitic in nature on roots only, makes surprisingly good progress in growth on water-congested tobacco leaves (Fig. 3, B). The endospores of this fungus are approximately the size of bacteria, suggesting that these may bodily penetrate the stomata and intercellular spaces before germination.

Infection with such leaf-parasitic fungi as *Helminthosporium sativum* and *Septoria lycopersici*, not known to occur on tobacco, has been more con-

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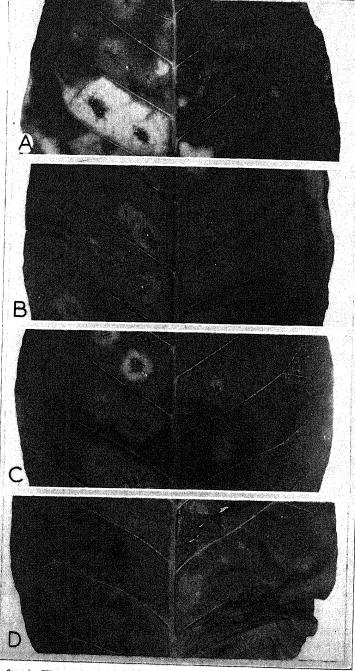


Fig. 3. A. Water-congested tobacco leaf, inoculated with Cercospora nicotianae. Wounded on left, not wounded on right side. B. Water-congested leaf inoculated with Thielaviopsis basicola. Needle scratches left side, needle pricks right side, unwounded far right (no infection). C. Localized water-congested areas produced by atomizing to varying degrees preceding inoculation with Cercospora nicotianae; far right, unconleaf with different treatments applied between lateral veins. At lower left wounded and unwounded areas are shown and at lower right wound inoculations with a toxin-free and a toxin-containing wildfire culture are shown.

spicuous on congested leaves than that from *Rhizoctonia sp.* and *Pythium sp.*, which normally occur on tobacco as stem parasites.

In contrast to considerable variability in stomatal penetration of congested leaves, the penetration following wounding occurs with certainty and regularity (Fig. 3, A). The removal of the normally present barrier to penetration in the epidermal layers in previously water-congested areas produces an unusually favorable situation for both penetration and infection. The conditions are essentially the same as for stomatal penetration of congested leaves except that the relatively large orifice of the wound permits the surface water on the wound, together with suspended particles, to gush immediately into the surrounding intercellular spaces for distances up to a centimeter or more. This action places a parasite, when present, in a location comparable to that of a weak culture solution in a microscopic-sized moist chamber, thus providing for limited multiplication and growth, which in turn results in disease expression in the host tissues.

In wounded-congested tissue, special interest surrounds the force which causes water and suspended substances to be drawn into the intercellular spaces with such rapidity and consistency as to suggest a reduced pressure in the intercellular spaces. Actually this force can be overcome by a maintained positive water pressure in the leaf of approximately the magnitude of guttation pressure, but there is no evidence that the inward force is of a true physiological nature. The movement of water and suspensions through wounds is also too nearly instantaneous to be attributed to simple diffusion, but is more likely related to surface tensions, or other attractions which in turn develop quick-acting, capillary forces through the stomatal openings and the narrow channels of the intercellular spaces. Almost identical behavior can be shown by touching a capillary tube containing water to the surface of India ink, or by connecting drops of ink and water on a glass slide by a narrow channel of water. Entrance of solutions or suspensions into the channels of the intercellular area is not only immediate but it may continue for a considerable period under proper conditions, so that succeeding drops applied may often be observed to be slowly drawn into the orifice in the epidermis.

Congestion with External Water Pressures

In some respects there is little essential difference between internally produced water congestion and "storm water soaking" brought on by external water pressure (1). Both forms may fill large or small areas of intercellular spaces with water (Fig. 1, B) and, while part of the storm water soaking results from the forcible propulsion of water through stomata and through the wounds produced, this condition may induce further congestion by capillary force, as well, when surface water is present. The important difference in the two forms lies in the fact that storm-water-soaked areas are usually or probably wounded, thus most likely obscuring the part played by stomatal penetration and natural water congestion when occurring together.

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In the present series of experiments, we have repeatedly produced various degrees of water soaking by atomizing the lower surface of detached leaves at a fairly constant water pressure for various lengths of time. Subsequent treatments with the various substances or inocula applied and other conditions of the test have been the same as for the controls and for leaves congested by internal water pressure. The degree of wounding, or effect on the cuticle and epidermis, may be often readily observed in such water-soaked, uninoculated areas, which eventually develop a blemish or "sheen" on the surface as frequently seen in field-grown crops following storms. The experimental irregularities and inconsistencies arise in efforts at producing water congestion of the external type with the minimum of injury to the cuticle. Leaves and parts of the same leaf vary greatly in the force required for the propulsion of water through stomatal openings. congestion occurring very easily in some instances without any visible evidence of surface injury. The best criterion yet found for determining the absence or presence of minute wounds in water-congested tissue is through the application of water-soluble dyes, such as rose bengal, to the treated area. If the dye enters immediately, wounds are present: if the dye enters slowly or not at all, the absence of wounds is indicated. When the atomizing is fairly heavy, yielding conspicuous, water-soaked areas with varying degrees of wounding of the epidermis, the results (Table 1), as might be expected, are almost identical with those of wounded, internally congested tissue (Fig. 3, B). The results from light atomizing were variable or irregular, probably because of the variation between no wounding and slight wounding. Although stomatal penetration may occur in such stormwater-soaked areas, there is naturally less certainty of the actual path of entry than in internally water-congested tissues (2). Also, there is no certainty that the natural protective cuticle or coatings on the leaf surface are not altered by any force of water sufficient to cause congestion when applied externally.

DISCUSSION

A series of investigations has been undertaken in this laboratory for the purpose of explaining the frequently observed erratic behavior of infection and disease. The predisposing influence of natural water congestion in the tissues has previously been emphasized (4). It was nevertheless clear that all water-congested tissues were not necessarily predisposed to infection, and that the occurrence of natural water congestion is a complex phenomenon involving many physiological factors in plants.

Infection studies on detached leaves, as reported in this paper, appeared to offer opportunities to test some of the more simple principles involved in infection. Substantially similar results have been secured on growing plants following natural water congestion. As the efforts progressed, however, it soon became evident that a wide variety of more detailed studies on growing plants should be performed and that the results with detached leaves were only of preliminary nature. Among these have been, for ex-

ample, the important differences that exist between outdoor and glasshouse-grown plants with respect to predisposition to infection. It has also been made increasingly obvious that leaves in a normal condition possess a remarkable degree of protection from penetration in their epidermal coatings, but that this may be greatly altered by nutrition and environment and often eliminated by wounding. When the epidermal barriers have been penetrated and the intercellular spaces invaded, infection is almost certain with normal parasites and is often likely with nontypical parasites and many saprophytes.

Of special interest is the relation of water congestion to physical penetratration, the natural variability in species and varieties (3) to this phenomenon, and the behavior of stomatal or other natural openings with respect to their influence on the penetration of parasites into the water-congested intercellular spaces.

SUMMARY

The mechanism of infection in detached, artificially water-congested leaves of tobacco and other plants was studied in refrigerator pans used as moist chambers. A considerable number of different kinds of organisms were used including both parasitic and saprophytic bacteria and fungi as well as other substances such as toxins, viruses, India ink, and dyes.

The individual leaves were congested artificially by internal water pressure followed by the application of the inocula in various ways on wounded and unwounded surfaces, in comparison with adequate controls. The treated leaves were then maintained in approximately normal condition in the moist chambers up to 6 to 12 days if necessary.

Penetration did not occur through normal (i.e., uncongested), unwounded tissues. Rare exceptions occurred with certain fungi such as Cercospora nicotianae but only after prolonged periods of incubation. Stomatal penetration frequently occurs through unwounded, water-congested tissues without the intervention of any injury to the cuticle or any exterior propulsion of the inoculum. Bodily penetration through the stomatal openings is dependent upon the presence of congestive water and leaf-surface water, with particles of sufficiently small size to pass through the openings. The mechanism involved is the establishment of a capillary force through the formation of a channel of water between leaf-surface water and the congestive water through which solutions or suspensions are transported.

Penetration in wounded plant tissues is usually immediate; and, when the leaf is also water congested, bacterial parasites in particular may be carried for considerable distances into the adjacent intercellular areas. In this location, the parasites find sufficient moisture and nutrients to multiply and hence cause disease in varying degrees depending in part upon the amount and period of water congestion in the tissues. Natural barriers to infection are destroyed by wounding and hence natural variations in resistance to disease may be impaired.

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These observations permit a better understanding of modified predisposition of plants to infection, and of the natural varietal variation in resistance of plants to disease.

WISCONSIN AGRICULTURAL EXPERIMENT STATION AND DIVISION OF TOBACCO INVESTIGATIONS. United States Department of Agriculture.

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PHYTOPATHOLOGICAL NOTES

The Relationship between American Tobacco Streak and Brazilian "Necrose Branca" or "Couve."—A virus disease resembling the American tobacco streak¹ was described from São Paulo, Brazil, under the names "necrose branca" (white necrosis) and "couve" (cabbage-leaved tobacco).² It was pointed out that the necrotic symptoms of this disease and the physical properties of the causal virus were similar to those of the American disease, but unlike tobacco streak, the necrotic symptoms were followed by incomplete recovery, and at this stage the affected plants showed leaf modifications (such as stiffness, narrowing, petiolated leaves in the case of sessile varieties, etc.), flowers sometimes with petals partly separated and the constant presence of a filament-like appendage at the extremity of each petal.

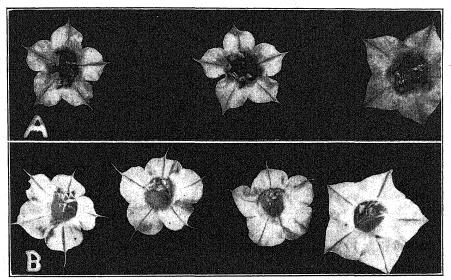


Fig. 1. A. Flowers from a plant affected by tobacco streak. Normal flower on right. Variety Turkish. (Photograph by J. A. Carlile.) B. Flowers from a plant affected by the Brazilian disease. Normal flower on right. Variety Bright Virginia.

During the writer's stay at the Rockefeller Institute for Medical Research, Princeton, N. J., in 1943, an attempt was made to prepare an antiserum to tobacco streak virus; this antiserum was to be taken to Brazil in order to test the serological relationship between the American and the Brazilian viruses, as the introduction of foreign diseased plant material for comparative studies was deemed inadvisable. Unfortunately, no active antiserum was produced and the relationship of the two viruses could not be tested by this method.

In the course of these studies it was noticed that plants of the Turkish

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¹ Johnson, J. Tobacco streak, a virus disease. Phytopath. 26: 285-292. 1936.

² Costa, A. S., A. R. Lima, and R. Forster. Necrose branca—uma moléstia de virus do fumo (*Nicotiana tabacum* L.) e "Fumo couve" como sintoma tardio. Jornal de

variety affected by tobacco streak had one characteristic feature of the Brazilian disease, hitherto not described in connection with the former: petals ending in a filament-like appendage. Figures 1, A, and 1, B, show this interesting symptom which furnishes additional evidence of relationship between the American and Brazilian viruses.—A. S. Costa, Department of Genetics, Instituto Agronômico de Campinas, São Paulo, Brazil.

Agar Medium and Technique for Isolating Pythium Free of Bacteria.— Isolation of Pythium from guayule seedlings (Parthenium argentatum Gray) affected with damping-off and seedling root rot by usual laboratory methods was generally unsatisfactory. The isolates when obtained in culture were usually contaminated with bacteria and the common practice of acidifying the agar medium to separate the fungus from the bacteria was never too satisfactory. In the course of investigational work on damping-off of guayule, a special agar medium and a technique was developed which facilitated the isolation of bacteria-free Pythium from diseased plants and from the soil. The method was particularly effective in isolating Pythium ultimum Trow., which has been reported as causing damping-off and a root rot of guayule.

This method has the advantage of yielding isolates of *Pythium* free of bacterial contamination from nonsurface-sterilized material. It also provides a simple and useful method of separating bacteria from *Pythium* and other fungi in bacterial contaminated cultures.

The formula for the agar medium is as follows:

Dextrose	10	Ω.
Ammonium acid phosphate (NH ₄ H ₂ PO ₄)		•
Potassium nitrate (KNO ₃)	1	g.
Magnesium sulphate (MgSO ₄)	1	g.
Agar	25	
Water, distilled	1000	cc.

The agar and dextrose were dissolved in 800 cc. of water by heating in a double boiler or autoclave. The other materials were dissolved in 200 cc. of water and added to the thoroughly dissolved agar and dextrose and autoclaved for 15 minutes at 15 pounds pressure. The usual procedure was to autoclave 1000 cc. of medium in three 500-cc. Erlenmeyer flasks. Each flask contained approximately 333 cc. of medium, and immediately after autoclaving the hot liquid medium was poured into sterile Petri dishes. However, the sterilized agar medium was occasionally stored in a refrigerator for several days or weeks. Liquefying the medium a second time by heating in a water bath did not adversely affect its qualities. The agar medium had a reaction of from pH 5.0 to 5.5.

The solidified agar medium, which was about 5 mm. thick in Petri dishes (9 cm. in diameter), was divided into 4 equal quarters with a small sterilized

¹ Campbell, W. A., and Bailey Sleeth. A root rot of guayule caused by *Pythium ultimum*. Phytopath. 35: 636-639. 1945.

metal spatula or wide scalpel. A small bit of inoculum consisting of diseased plant tissue or soil was placed in the center of each quarter section. Sometimes, as a matter of convenience, the inoculum was placed on the agar before the agar was quartered. Three of the sections were carefully lifted out of the Petri dish by means of a small sterilized flexible metal spatula and placed inverted in separate sterile Petri dishes. This part of the process was similar to that used in turning a pancake. The 4th or remaining section was inverted in the original Petri dish. A certain amount of care had to be exercised in inverting and placing the inoculated section of agar medium so that the inoculum would be completely covered with the agar block. An effort was made to create a seal around the inoculum by having the edges of the agar block come in contact with the bottom of the Petri dish.

If Pythium were present in the original inoculum, scattered hyphae grew through the agar block and reached the upper surface within 24 hours at laboratory temperature. If other slower growing fungi were present in the plated inoculum, hyphae of Pythium were the first to appear on the surface so that hyphal tip transfers usually resulted in pure cultures. When reisolations were made from infected seedlings in pathogenicity tests, it was not unusual to obtain bacteria-free Pythium in over 90 per cent of the attempts. The growth of bacteria was inhibited while Pythium and other fungi grew well on the special agar medium. Cultures of Pythium contaminated with bacteria were readily freed of bacteria by the same method.

In order to secure the best results, the agar sections should be fairly thick and the agar medium of sufficient hardness to permit lifting the sections without breaking. Since the quality of agar-agar varies, the amount in the formula may need to be varied accordingly in order to obtain the desired consistency of the agar medium.—Balley Sleeth, Special Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture, Salinas, California.

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ANNOUNCEMENT

AMERICAN PHYTOPATHOLOGICAL SOCIETY

Annual Meeting

The American Phytopathological Society will meet with the American Association for the Advancement of Science in St. Louis, Missouri, March 27–30, 1946. The headquarters hotel for the Society will be announced in the January issue of Phytopathology.

Members wishing to read papers or to give demonstrations should submit triplicate abstracts, not exceeding two hundred words, to the Office of the Secretary before January 25, 1946. The time required for reading papers, not to exceed fifteen minutes, projection apparatus needed, and section preference should accompany the abstract. Abstracts of demonstrations should state the floor and wall space required as well as the electrical and other equipment needed.

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Junior authorship indicated by pages in "(()")"

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Page 143, line 1, read Tilletia tumefaciens was founded by Sydow (See 5) for Tilletia tumefaciens Sydow

Page 145, add to literature cited, 5. Sydow, H. and P., and E. J. Butler. Fungi Indiae Orientalis. Pars IV. Ann. Mycol. Berl. 10: 244-280. 1912

ERRATA, VOLUME 35

Number 2, Table of Contents, read H. R. Rosen for H. Rosen

Page 119, item 9 in literature cited, read Root defects and fungi for Root defects

Page 129, line 4, read presumably for presumbaly

Page 187, line 5, read Romania for Roumania

Page 239, item 3 in literature cited, read 1919 for 1939

Page 315, item 7 in literature cited, read 1945 for 1944

Page 340, line 41, read Tong-kin for Tungkin

Page 341, line 14, read D. C. Edwards for E. J. Edwards

Page 545, line 16, read In 1941 the percentage for In 1939 the percentage

Page 558, line 27 and Table 3, Col. 4, line 2, read 26.9 for 19.7

Page 743, line 2, read Pellicularia for Pellicullaria

Page 767, line 17, read X217 for X216